

# BEST AVAILABLE COPY

Applicant : Carl-Axel Bauer *et al.*  
Serial No. : 10/010,283  
Filed : November 13, 2001  
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Attorney's Docket No.: 06275-150003 / D 1841-3P US

by Giardina *et. al*” and (II) “each of [the] active agents (budesonide and formoterol) utilized in Carling’s medicament are individually known to treat COPD conditions.”

Before addressing these contentions, however, Applicants first note that Andersson *et al.* (U.S. Patent No. 6,598,603 B1) is not prior art against Applicants’ claims, because the earliest date as of which this patent is prior art (*i.e.*, under 35 USC §102(e)) is December 31, 1997, which is later than the priority date of the instant application (September 19, 1997; a copy of this priority document (SE 9703407-8) and other references cited below are enclosed herewith as Exhibit A. A certified copy of the Swedish priority document will follow under separate cover.). Thus, citation of this reference is improper. Furthermore, even if it were citable as prior art, Applicants disagree with the Examiner’s conclusions as to the teachings of this reference, as will be discussed below.

## I. Non-Obviousness of Employing the Carling Medicament in the Treatment of COPD

The obviousness rejection appears to hinge on the Examiner’s assertion that “COPD and asthma are well known by Giardina *et al.* as respiratory disorders” (Office Action, p. 5), and thus Applicants will first discuss the relevance of the newly-cited Giardina reference to Applicants’ previous arguments concerning Carling.

The Giardina reference does in fact refer to both asthma and COPD under the umbrella term of “respiratory disorders.” It also includes “airway hyperreactivity” and “cough” within this term (col.1, lines 37-39). There is no question that the term “respiratory disorders” can be read broadly to include a long list of unrelated conditions. Indeed, Applicants submitted evidence to that effect in the previous response (see the discussion of the Gibson *et al.* “Respiratory Medicine” textbook at page 2 of the Declaration of Dr. Jan Trofast submitted with the response filed March 1, 2004 (the “Trofast Declaration”)). The Giardina *et al.* reference cited by the Examiner is no more relevant to that point than is the Gibson reference. The pertinent question is not whether it is possible to find a definition of “respiratory disorders” that encompasses COPD: that is a given. Instead, one should ask, “What would one of ordinary skill in the field of treating COPD have read the term ‘asthma and other respiratory disorders’ to mean

in the context of the Carling *et al.* reference?" It would be ridiculous to say it means "asthma and all other respiratory disorders," as there is no reason to expect the drug combination taught by Carling *et al.* to have any value for treating such widely disparate respiratory disorders as, say, lung cancer, adult respiratory distress syndrome, or cough. A person who knew anything about how formoterol and budesonide function would realize this. A more reasonable reading of the phrase in Carling *et al.* would be one that is relevant to the context of Carling *et al.*, *i.e.*, limited to asthma and asthma-like conditions. This is a point made in the Trofast Declaration, a point that the Examiner cannot rebut simply by finding yet another broad definition of "respiratory disorders" somewhere in the art. Unlike Carling *et al.*, Giardina *et al.* was not focused at all on asthma or any other particular condition (respiratory or otherwise). Rather, Giardina *et al.* proposed using tachykinin receptor antagonists to treat an extraordinarily wide variety of conditions, most of which have nothing to do with the respiratory system (see col. 1, line 37 through col. 2, line 17). Thus, Giardina *et al.* used the term "respiratory diseases" in a context quite different from the very focused context of Carling *et al.*, and that would be immediately apparent to one of ordinary skill. As explained in the Trofast declaration, taking into account the context of the Carling *et al.* reference, the phrase "other respiratory disorders" in Carling *et al.* "would have been understood to refer to respiratory disorders similar to asthma, i.e. mainly of bronchospastic nature" (page 2, section 5 of the Declaration; emphasis in original).

In her "Response to Arguments," the Examiner summarily dismisses Dr. Trofast's interpretation of Carling *et al.*, without articulating any adequate reasons for disregarding the evidence of an expert in the field of respiratory disorders. Such a failure to consider the totality of the record, by disregarding evidence that directly addresses the issue of patentability, is clearly improper. See, e.g., In re Alton, 76 F.3d 1168, 1174-1176 (CAFC 1996).

In support of her assertion that the Trofast declaration is not persuasive, the Examiner states only that "COPD and asthma are well known by Giardina *et al.* as respiratory disorders" and again that "COPD is [a] well known respiratory disorder along with asthma by Giardina *et al.*" This is not an adequate basis for disregarding Dr. Trofast's expert testimony. While "respiratory disorders" in some contexts may well include COPD (and tuberculosis and lung

cancer and cough and all of the other conditions listed in Gibson *et al.* as cited in the Trofast declaration), that does not mean that one of ordinary skill would read all of those conditions into the phrase as used in the Carling *et al.* reference. Dr. Trofast explained why the many respiratory conditions listed in the Gibson textbook are not relevant to the Carling *et al.* disclosure. In response, the Examiner merely cites another prior art list of respiratory diseases, providing no rationale as to why this list is any more relevant than the Gibson list and no explanation as to why Dr. Trofast's comments regarding the Gibson list of respiratory disorders are not equally applicable to the list in the Giardina *et al.* patent. Applicants therefore respectfully request that the Examiner reconsider Dr. Trofast's comments from the March 1, 2004, declaration and either withdraw the Giardina *et al.* reference with respect to the obviousness rejection or explain why she considers Dr. Trofast's comments to be inapplicable to Giardina *et al.*

There is also ample evidence that proven asthma therapies, such as the medicaments described by Carling *et al.*, cannot be expected to successfully treat non-asthma-related categories of respiratory diseases. Such evidence includes, for example, Engel *et al.* (*Eur. Respir. J.* 2:935-939, 1989), and Watson *et al.* (*Chest* 101:350-55, 1992), submitted herewith. Engel *et al.* reports that budesonide did not improve symptom scores, ventilatory capacity, or airway responsiveness in subjects with chronic bronchitis (see the abstract), while Watson *et al.* reports that inhaled budesonide failed to improve symptoms in middle-aged male smokers with mild airway obstruction (see the abstract). These are but a few of the many examples of disorders that fall in the broad category of "respiratory disorders" but do not respond to treatment with budesonide, despite the demonstrated efficacy of this compound to treat asthma.

## II. Budesonide Was Not Known to Treat COPD

The Examiner also alleges, in support of the rejection, that "each of [the] active agents utilized in Carling's medicament are individually known to treat COPD conditions." Applicants strongly disagree with this assertion. At the time of Applicants' invention, budesonide was not believed to be suitable for treatment of COPD.

The Examiner relies on Andersson *et al.* as evidence that budesonide was known at the time of the invention as an effective treatment for COPD. Even if this reference could properly be cited as prior art, which it cannot for the reasons discussed above, it would not provide such evidence. The Andersson *et al.* patent states that budesonide can be used to treat respiratory disorders such as asthma, COPD, bronchiolitis, croup, and bronchopulmonary dysplasia, disorders that fall under the broad category of respiratory disorders described in the Trofast declaration. However, data presented in the Andersson *et al.* reference shows only that budesonide can be used to treat asthma. Proper application of an obviousness rejection requires that there have been a reasonable expectation of success in the prior art. In light of the multitude of evidence in the scientific literature showing that budesonide does not significantly reduce the symptoms of COPD<sup>1</sup>, the artisan, reading Andersson *et al.*, would not have had a reasonable expectation that budesonide could be used to successfully treat COPD, nor would Andersson *et al.* have led the artisan to have such an expectation. The mere mention of this disorder as part of a generic list in the Andersson *et al.* patent, unaccompanied by any experimental evidence relevant to treatment of COPD, is not sufficient to rebut the substantial evidence that budesonide is NOT useful for treating COPD. Because none of the prior art (including Andersson *et al.*) establishes a reasonable expectation of success that the claimed invention would work, the Examiner has not made out a prima facie case of obviousness.

As further evidence that budesonide was not believed at the time of the invention to be effective in treating COPD, Applicants enclose Keatings *et al.* (*Am. J. Respir. Crit. Care Med.* 155:542-548, 1997). Keatings *et al.* reports that in COPD patients, inhaled budesonide resulted in no clinical benefit in either lung function or symptom scores, and no significant change in the inflammatory indices assayed (see the abstract at page 1). These results were consistent with studies with oral prednisolone, a glucocorticoid steroid similar to budesonide. The authors concluded, "...it is clear that the inflammatory mechanisms in COPD differ significantly from those in asthma" (page 547, final paragraph).

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<sup>1</sup> See, e.g., Carling *et al.*, discussed at page 4 of the submission mailed March 1, 2004; Buist and Calverley *et al.*, discussed at pages 4 and 5 of the Trofast declaration mailed March 1, 2004; and *Nederland Tijdschrift Voor Geneeskunde* discussed at page 4, and Pauwels *et al.* and the Neiwoehner study discussed at page 5 of the Appeal Brief mailed March 26, 2003.

Applicants maintain that Examiner has failed to produce evidence of the successful treatment of COPD with budesonide alone, and further that the Examiner has failed to show that it would have been obvious from the prior art to combine budesonide with formoterol to treat COPD.

While Cazzola does teach the use of formoterol to treat COPD, this teaching would not have suggested the use of the budesonide/formoterol combination to treat COPD, in view of the overwhelming evidence that budesonide would not be successful in treating COPD.

Objective evidence, including unexpected or surprising results, must also be considered with respect to the issue of obviousness, and when any of this evidence is submitted, the Examiner must evaluate the evidence. See Graham v. John Deere, 38 U.S. 1 (1966). See also Stratoflex, Inc. v. Aeroquip Corp., 713 F.2d 1530 (Fed. Cir. 1983) ("Evidence arising out of secondary considerations must always when present be considered en route to determination of obviousness of a patent, and it is error to exclude that evidence from consideration.").

Applicants remind the Examiner of the unexpected synergistic effects of budesonide and formoterol on the treatment of COPD described in the declaration by Christer Hultquist, M.D., submitted December 13, 2002 (hereafter, "the Hultquist declaration"). According to the Hultquist declaration, treatment of COPD patients with formoterol alone increased the rate of severe exacerbations by about 3% as compared to treatment with placebo, and treatment with budesonide alone decreased the rate of severe exacerbations by 12% as compared to treatment with placebo. Neither of these values was statistically significant. However, treatment with the combination therapy unexpectedly reduced the rate of severe exacerbations by 24% as compared with placebo, indicating a synergistic effect of the combination therapy. This rate of reduction was statistically significant ( $p < 0.05$ ). Similarly, a synergistic effect on morning peak expiratory volume (PEF) was observed following administration of the budesonide/formoterol combination therapy: administration of formoterol alone resulted in an adjusted mean change of morning PEF, as compared to placebo, of 11.1 L/min; administration of budesonide alone resulted in an adjusted mean change of morning PEF, as compared to placebo, of 3.5 L/min; and administration of the budesonide/formoterol combination resulted in an adjusted mean change of morning PEF,

as compared to placebo, of 18.3 L/min, which is notably higher than an additive result.

Applicants request that the Examiner reconsider the Hultquist declaration with respect to the obviousness rejection.

Applicants also note that the Examiner does not seem to have considered Calverley *et al.* (*Eur. Resp. J.* 22:912-919, 2003) submitted with the Request for Continued Examination (RCE) filed March 1, 2004. As discussed in the arguments filed with the Request, Calverley *et al.* reports the successful treatment and prevention of exacerbations in COPD patients using a combination of budesonide and formoterol. As described previously, Table 3 of Calverley *et al.* shows that treatment with the combination reduces the number of exacerbations more effectively than either budesonide or formoterol alone. As reported in the table, the number of exacerbations per year (mean rate per patient per year) was 1.80 during treatment with a placebo and a near-equivalent 1.85 during treatment with formoterol alone. Exacerbations were mildly reduced following treatment with budesonide (1.60 mean rate per patient per year) although this decrease was still not significant compared to treatment with placebo. Treatment with the combination of budesonide and formoterol reduced the rate of exacerbations to 1.38, a significant reduction as compared to treatment with placebo ( $p=0.029$ ). This result was surprising given the low efficacy or ineffectiveness of treatment with either budesonide or formoterol alone. The authors added that “[i]t...seems that formoterol and budesonide in combination are more effective at reducing proliferation of airway smooth muscle than either drug alone, as a result of synchronised cellular signaling...” (page 918, column 2). Applicants request that the Examiner consider these unexpected results as well as those presented in the Hultquist declaration.

Given the controversy at the time the application was filed regarding the efficacy of budesonide alone in the treatment of COPD, as evidenced by the art presented previously and with this response, and given the unexpected findings of a synergistic effect upon using the budesonide/formoterol combination therapy, it would not have been obvious to treat COPD with a combination of these compounds. The Examiner concludes at page 4 of the Office Action that “[a]bsent any evidence to [the] contrary, there would have been a reasonable expectation of

successfully treating COPD by employing Carling's formulation to achieve greater efficiency and duration of action..." The evidence presented herewith (and with previous responses) is indeed "evidence to the contrary." The totality of the record indicates that, at the very least, there was serious doubt among those working in the field of respiratory diseases at the time the application was filed as to the efficacy of budesonide alone for the treatment of COPD. The record also clearly indicates that those working in the field appreciated the differences in the inflammatory mechanisms of, and hence the appropriate treatments for, asthma and COPD. Accordingly, Applicants maintain that at the time the invention was made there would not have been a reasonable expectation of success regarding the treatment of COPD with a combination of budesonide and formoterol. That makes the synergistic results described above even more surprising, and even stronger evidence of nonobviousness.

In view of the statements above, Applicants request that the Examiner withdraw the rejection of claims 9, and 11-25 under 37 C.F.R. §103(a) as being unpatentable over Carling *et al.*, in view of Cazzola *et al.* and Andersson *et al.* and further in view of Giardina *et al.*

Applicants further request that the Examiner consider the references cited in the Form PTO-1449 originally submitted with the application on November 13, 2001, and submitted a second time by facsimile on July 19, 2002. Examiner noted on a copy of the Form PTO-1449 returned to Applicants on January 29, 2002, that references AF-AQ were not provided. Applicants note that reference AF was originally cited by the Examiner in the U.S. priority application 09/194,290 (hereafter the '290 application), filed November 23, 1998, and reference AG was originally submitted by Applicants in the '290 application. Documents AH-AQ were originally submitted by Applicants in U.S. priority application 09/670,457, filed September 26, 2000. Applicants therefore request that the Examiner consider these references and return an initialed copy of the Form PTO-1449. New copies of these references will be provided if necessary.

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Enclosed is a \$1,020 check for a Petition for Extension of Time for three months. Please apply any other charges or credits to deposit account 06-1050, referencing Attorney Docket Number 06275-150003.

Respectfully submitted,

Date: December 20, 2004

Allyson Hutton Reg. No. 54,154  
for Janis K. Fraser, Ph.D., J.D.  
Reg. No. 34,819

Fish & Richardson P.C.  
225 Franklin Street  
Boston, MA 02110-2804  
Telephone: (617) 542-5070  
Facsimile: (617) 542-8906

Doc. No. 20996053



## A trial of inhaled budesonide on airway responsiveness in smokers with chronic bronchitis

T. Engel, J.H. Heinig, O. Madsen\*, M. Hansen\*, E.R. Weeke\*

*A trial of inhaled budesonide on airway responsiveness in smokers with chronic bronchitis. T. Engel, J.H. Heinig, O. Madsen, M. Hansen, E.R. Weeke.*

**ABSTRACT:** The aim of the present randomized, double-blind study was to evaluate the effect of inhaled budesonide on daily symptoms, ventilatory capacity, and airway responsiveness in smokers with chronic bronchitis. Twenty five subjects with a provocative concentration producing a 20% fall in forced expiratory volume in one second  $PC_{20}$  ( $FEV_1$ ) less than 2.0  $mg \cdot ml^{-1}$ , by bronchial histamine challenge, were included. Eighteen subjects accomplished the entire 12 week study, eight receiving inhaled budesonide 400  $\mu g$  *b.i.d.* and ten receiving placebo. Cough decreased significantly in the actively treated group during the treatment period, but no change could be demonstrated in expectoration, dyspnoea, or sleep disturbances. No changes in any of these symptoms were found in the placebo group, and no differences in symptoms scores were found between the groups. No significant differences in ventilatory capacity or bronchial responsiveness could be demonstrated. In conclusion, a moderately high dose of inhaled steroid in eight subjects with chronic bronchitis did not improve the symptom scores, ventilatory capacity, or airway responsiveness to any clinically relevant degree.

*Eur Respir J.*, 1989, 2, 935-939.

Allergy Unit TTA 7511, State University Hospital, Copenhagen, Denmark.

\* Allergy Clinic and Medical Dept, County Hospital, DK-4000 Roskilde, Denmark.

Correspondence: Dr T. Engel, Allergy Unit TTA 7511, State University Hospital, Tagensvej 20, DK-2200 Copenhagen, Denmark.

**Keywords:** Bronchial challenge; budesonide; chronic bronchitis; histamine; hyperresponsiveness; steroid; tobacco; ventilatory capacity.

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One of the characteristic features of asthma is the increased sensitivity of the airways to extrinsic stimuli, e.g. cold air or histamine [1], which can be reduced by steroids [2]. Bronchial hyperresponsiveness is also known to occur in subjects with chronic bronchitis and normal ventilatory capacity, although to a minor degree [3-8]. Earlier studies of subjects with chronic bronchitis have investigated the efficacy of steroids in severe bronchial obstruction [9, 10]. We have been unable to find any published detailed results on the effect of inhaled steroids on hyperresponsiveness in subjects with chronic bronchitis and normal ventilatory capacity. However, a recently published abstract [11], showed no effect of budesonide.

The aim of the present study was to evaluate the effect of inhaled budesonide 400  $\mu g$  *b.i.d.* on daily symptoms, ventilatory capacity, and airway responsiveness in subjects with chronic bronchitis, forced expiratory volume in one second ( $FEV_1$ ) above 70% predicted, and bronchial hyperresponsiveness.

### Materials and methods

#### Design

The study was performed in a randomized, double-blind, placebo-controlled fashion. After a 2 week run-in period, the subjects were randomized to receive either

budesonide 400  $\mu g$  *b.i.d.* by inhalation through a 750 ml spacer (Nebuhaler®), or placebo canisters which appeared identical, during a 12 week study period (fig. 1). The subjects registered severity of the following symptoms daily in a diary: cough, dyspnoea, sputum, and nightly sleep disturbances due to pulmonary causes (table 1). Peak expiratory flow (PEF) was measured twice daily, throughout the run-in period and the entire 12 week study period, on a mini Wright peak flow meter (Airmed, Clement Clarke International Ltd, London, UK), the best of three attempts being recorded. Measurements of ventilatory capacity and bronchial histamine challenge were performed every fourth week during the study period (fig. 1).

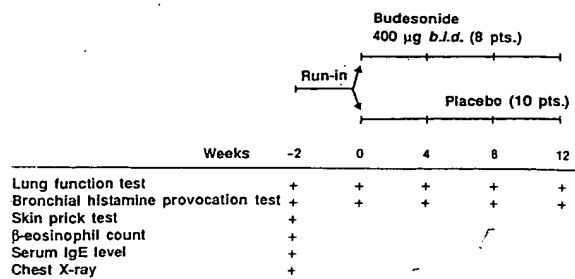


Fig. 1. - Study design.

Table 1. — Symptom scores recorded in diaries

Symptom score:	0	1	2	3
Cough	None	Few coughs every day	Repeated cough attacks, but only in the morning or during the day	Persistent cough attacks during the day and night
Expectoration	None	Sputum easily expectorated	Difficulties in bringing up sputum	Impossible to bring up sputum
Dyspnoea	None	Dyspnoea from climbing one floor	Dyspnoea from walking on plain level	Dyspnoea at rest
Nightly sleep disturbance due to pulmonary causes	None	Awake once per night	Awake 2-3 times per night	Awake $\geq 4$ times per night

Table 2. — Descriptive data prior to the run-in period for the 18 subjects

	Budesonide	Placebo
Number of subjects	8	10
Age yrs	50.9 (37-57)	49.9 (43-58)
Sex M/F	2/6	6/4
Tobacco pack-years	33.3 (23-53)	36.6 (19-54)
FEV <sub>1</sub> l	2.32 (1.57-3.06)	2.81 (1.73-4.88)
FEV <sub>1</sub> % pred	96.9 (74-128)	97.7 (78-126)
FEV <sub>1</sub> /FVC %	72 (56-95)	73 (55-84)
PEF home measurements* % pred (morning)	82.6 (58.5-130.1)	89.4 (78.1-99.5)
PEF home measurements* % pred (evening)	87.2 (55.8-118.3)	91.3 (75.2-104.6)
PC <sub>20</sub> (FEV <sub>1</sub> ) mg·ml <sup>-1</sup>	0.54 (0.23-1.10)	0.75 (0.20-1.65)

All data are arithmetic means except PC<sub>20</sub> data, which are geometric means (range in parentheses). \*: arithmetic mean of the 2 week run-in period; FEV<sub>1</sub>: forced expiratory volume in one second; FVC: forced vital capacity; PEF: peak expiratory flow; PC<sub>20</sub>(FEV<sub>1</sub>): provocative concentration producing a 20% fall in FEV<sub>1</sub>.

### Subjects

Twenty five subjects with chronic bronchitis, defined as cough and expectoration for at least three months a year during at least the preceding two years [12], and

moderate to severe bronchial hyperresponsiveness as judged by a bronchial histamine challenge (provocative concentration producing a 20% fall in forced expiratory volume in one second PC<sub>20</sub>(FEV<sub>1</sub>)  $\leq 2.0$  mg histamine·ml<sup>-1</sup>) were selected from a previous study [3]. All subjects were current tobacco smokers, 30-60 yrs of age, with a daily consumption of at least five cigarettes or a corresponding amount of pipe tobacco, cigarillos or cigars. All had an FEV<sub>1</sub>  $\geq 70\%$  predicted [13] and normal chest X-ray. None had a history of asthma as defined by SCADDING [14] or allergic rhinitis, or had had an airway infection during the last six weeks. None had a positive skin prick test with the ten most common inhalant allergens in Denmark (SoluPrick®, ALK, Copenhagen, Denmark), [15], elevated number of blood eosinophils ( $>400 \times 10^6 \cdot l^{-1}$  whole blood), or increased plasma immunoglobulin E (IgE) ( $>100$  kU·l<sup>-1</sup>). None had ever been treated with inhaled or systemic glucocorticoids.

Four subjects were excluded during the run-in period because of lack of time or failure to attend the clinic at the scheduled time, and two for other reasons. Nineteen subjects completed the study. The weight of the returned medication canisters indicated that one subject had used less than half of the prescribed dose, and this subject was, therefore, omitted from the statistical analysis. The remaining subjects had used the recommended dose of study medication. The material, therefore, consists of 18 subjects, eight receiving budesonide and ten placebo (table 2).

Subjects were included in the study during the months December-March, and were thus followed until June. During the study, one subject in the placebo group experienced an exacerbation which was treated with mucolytics. The subject continued taking the study medication during the exacerbation; histamine challenge was not performed until six weeks after the exacerbation.

### Ventilatory capacity

Measurement of the ventilatory capacity included forced expiratory volume in one second (FEV<sub>1</sub>) and forced vital

capacity (FVC), and was carried out on a recently calibrated dry-wedge spirometer (Vitalograph Ltd, Buckingham, UK). All manoeuvres were repeated until three consecutive measurements showed a variation of 5% or less, or a maximum of eight attempts was reached. The largest value obtained was used in the subsequent analysis.

### Bronchial histamine challenge

Tobacco was withheld prior to the challenge as recommended by the Societas Europaea Physiologiae Clinicae Respiratoriae (SEPCR) working group [16]. No subject used any medication known to interfere with the bronchial histamine challenge during the study period. The challenge was performed with a jet nebulizer-Pari 'halier boy, airflow 11 l·min<sup>-1</sup>, output 0.27±0.03 ml·min<sup>-1</sup> (mean±sd) (measured at the Allergy Clinic), particle size 0.5–5.5 µm (manufacturer's declaration). Inhalations were performed for 2 min with a 5 min interval. After inhaling isotonic saline, the subjects inhaled increasing doses of unbuffered histamine dihydrochloride, alternating with measurement of the ventilatory capacity 90 s after termination of inhalation. If a 20% decrease in FEV<sub>1</sub> had not yet been obtained, the test was discontinued after inhalation of histamine dihydrochloride 8 mg·ml<sup>-1</sup> [17]. The result was expressed as the concentration of histamine causing a 20% decrease in FEV<sub>1</sub>, compared to the FEV<sub>1</sub> value after inhaling isotonic saline (PC<sub>20</sub>FEV<sub>1</sub>) using interpolation on the log dose response curve. Values above 8 mg·ml<sup>-1</sup> were reported as 8 mg·ml<sup>-1</sup>, no values below 0.125 mg·ml<sup>-1</sup> were obtained.

### Statistics

Unless otherwise stated, all results shown are arithmetic mean±sd. Statistical analysis was performed using Student's t-test, paired or unpaired as appropriate. All PC<sub>20</sub> (FEV<sub>1</sub>) values were logarithmically transformed prior to statistical analysis.

### Results

Cough decreased significantly in the budesonide group after the 8–12 week study period when compared to the run-in period ( $p<0.05$ , paired t-test) (fig. 2). No differences in the placebo group were found ( $p>0.05$ , paired t-test), and no differences between the budesonide and the placebo groups could be demonstrated ( $p>0.05$ , unpaired t-test). None of the remaining symptoms, sputum, dyspnoea, or nightly disturbances from pulmonary causes, showed significant differences between the groups ( $p>0.05$ , unpaired t-test) or changes as compared to the run-in period ( $p>0.05$ , paired t-test).

There was no significant difference in FEV<sub>1</sub>% between the budesonide and the placebo groups prior to the start of therapy or after 4, 8 and 12 weeks' study period ( $p>0.05$ , unpaired t-test) (fig. 3). No changes in either

the budesonide or the placebo groups during the study could be demonstrated ( $p>0.05$ , paired t-test). Home peak expiratory flow (PEF) measurements (% predicted) showed no differences between the groups ( $p>0.05$ , unpaired t-test) (fig. 4). No subjects demonstrated a 20% or greater daily fluctuation in home measurement of PEF.

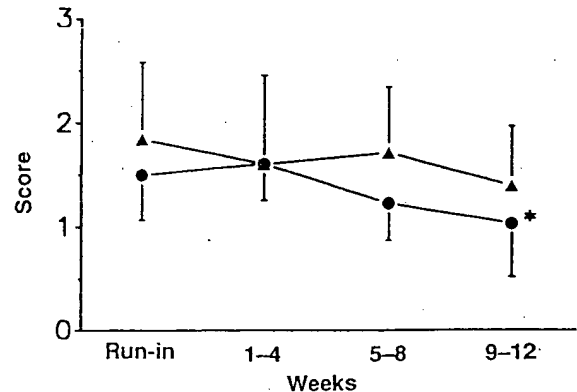


Fig. 2. – Cough symptom scores in 18 subjects with chronic bronchitis, treated with budesonide (●) or placebo (▲), arithmetic mean and sd (error bars). \*: different from the run-in period ( $p<0.05$ ).

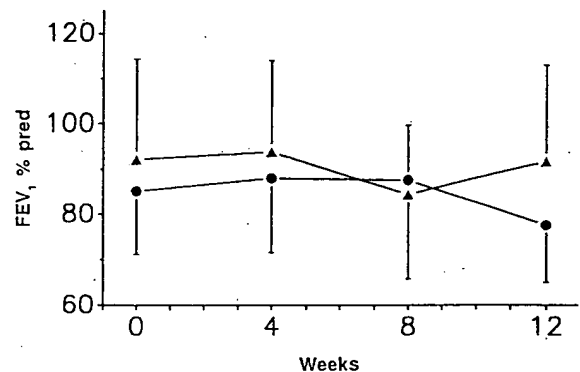


Fig. 3. – Ventilatory capacity (FEV<sub>1</sub> % pred) (mean±sd) in 18 subjects with chronic bronchitis, treated with budesonide (●) or placebo (▲). FEV<sub>1</sub>: forced expiratory volume in one second.

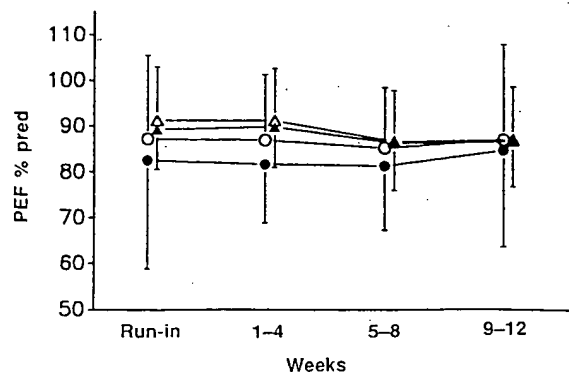


Fig. 4. – Home PEF measurements (% pred), best of three attempts, arithmetic mean±sd. Circles: budesonide group; triangles: placebo group; filled symbols: morning values; open symbols: evening values; PEF: peak expiratory flow.

Comparison between the budesonide and the placebo groups revealed no difference in  $PC_{20}(FEV_1)$  before the start of therapy or after 4, 8, and 12 weeks' study period ( $p > 0.05$ , unpaired t-test) (fig. 5). Neither the budesonide group nor the placebo group showed significant alterations in  $PC_{20}(FEV_1)$  after 4, 8 and 12 weeks' therapy (all  $p > 0.05$ , paired t-test).

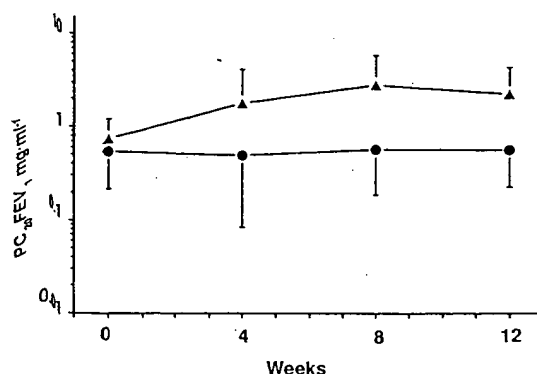


Fig. 5. Degree of bronchial responsiveness,  $PC_{20}(FEV_1)$ , (mean  $\log_{10}$ , bars indicate SD of  $\log_{10}$ ) in the 18 subjects with chronic bronchitis, treated with budesonide (●) or placebo (▲).  $PC_{20}(FEV_1)$ : provocative concentration producing a 20% fall in forced expiratory volume in one second.

### Discussion

Treatment with inhaled glucocorticoids is well-established in chronic asthma, where it has been shown to reduce the nonspecific bronchial hyperresponsiveness [2, 18, 20]. However, not all asthmatics respond equally well to steroid therapy [21]. Steroids are thought to reduce the inflammation in the bronchial wall of the asthmatic, although the mechanisms by which they act are still uncertain [22].

Eight to twelve weeks after the start of therapy we found a slight, beneficial effect of budesonide with respect to cough compared to the run-in period. Cough was the only symptom which decreased significantly.

Measurements of ventilatory capacity performed at the Allergy Clinic ( $FEV_1$ , FVC) and home measurements of PEF showed no improvement and no difference between the groups.  $FEV_1$  was above 70% in all subjects prior to start of the study, and mean PEF was above 70% during the run-in period in all but three subjects in the budesonide group. Increases could, therefore, hardly be expected. There was a trend towards higher values for  $FEV_1$  and PEF in the placebo group at inclusion, which was, however, abolished when calculated as % predicted; this was probably due to the high percentage of women in the budesonide group. These results parallel those recently published by WATSON *et al.* [11].

Neither the budesonide nor the placebo group showed any significant improvement with respect to bronchial responsiveness. The dose of budesonide given was higher than that given to asthmatics in earlier studies, where an effect was demonstrated [2, 19, 20], so underdosing is

not likely to have occurred. Only 18 of the 25 subjects accomplished the entire study, and bias in the selection may, therefore, occur. No significant differences between the subjects accomplishing the study and the drop-outs were registered regarding bronchial responsiveness,  $FEV_1$  % predicted, smoking habits, age and sex. Increasing the number of subjects is not likely to change the results, demonstrating an effect of inhaled budesonide on hyperresponsiveness, since only the placebo group showed a trend towards decreased responsiveness ( $p=0.055$ ) (fig. 5).

In order not to overlook any possible effect, the study period was chosen as 12 weeks. The great variances in symptoms over the seasons of the year and the risk of exacerbations would probably make the results of a 24 week cross-over design unreliable. Our study period ran into the spring, which might explain the trend towards decreased responsiveness in the placebo group. This does not, however, explain why we found no effect on ventilatory capacity or hyperresponsiveness in the budesonide group.

The pathogenesis of hyperresponsiveness in asthma and chronic bronchitis may be of a different nature. Chronic bronchitis is dominated by hyperplasia of goblet cells and mucous glands, partial bronchial obstruction due to sputum and epithelial hyperplasia, and a reduced number of cilia. Steroids are reported not to have any effect on mucociliary clearance in chronic obstructive bronchitis [23].

In conclusion we found a decreased cough score in eight subjects treated with inhaled budesonide 800  $\mu$ g daily for 12 weeks. No clinically relevant differences in ventilatory capacity,  $PC_{20}(FEV_1)$ , home measurement of PEF, or symptom scores regarding dyspnoea, sputum, and nightly sleep disturbances were found. Our results, therefore, do not support the use of inhaled steroids in subjects with chronic bronchitis,  $FEV_1$  above 70%, and bronchial hyperresponsiveness, unless cough is a predominant symptom.

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- RÉSUMÉ: Une hyperréactivité bronchique, exprimée en PC<sub>20</sub> VEMS après provocation par inhalation d'histamine, a été retrouvée chez 52 de 95 fumeurs dont le VEMS est supérieur à 70% des valeurs prédites, mais qui sont atteints de bronchite chronique. Le degré de réactivité était systématiquement inférieur à celui trouvé chez les asthmatiques paires, mais nettement supérieur à celui observé chez les sujets normaux. Le degré d'hyperréactivité est en corrélation significative avec les valeurs de base de la capacité ventilatoire, l'âge et la consommation de tabac, mais sans rapport avec le sexe. PC<sub>20</sub> MEF<sub>50</sub> a montré le même type de distribution que PC<sub>20</sub> VEMS, mais n'a pas ajouté d'information complémentaire. La pente de la courbe dose-réponse du MEF<sub>50</sub> n'a de corrélation avec aucun des paramètres mesurés. La pente des courbes dose-réponse du VEMS, par contre, montre une corrélation significative avec la consommation de tabac. Il y aurait lieu d'investiguer dans quelle mesure le degré d'hyperréactivité bronchique pourrait constituer un indice d'incapacité future.
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# Effects Of Inhaled and Oral Glucocorticoids on Inflammatory Indices in Asthma and COPD

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VERA M. KEATINGS, ANON JATAKANON, Y. MIIN WORSDELL, and PETER J. BARNES

Department of Thoracic Medicine, National Heart and Lung Institute, London, United Kingdom

The role of glucocorticoids in the treatment of chronic obstructive pulmonary disease (COPD) is controversial. We have previously described high numbers of neutrophils and high concentrations of the inflammatory cytokines interleukin-8 (IL-8) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and of the cell activation markers eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), myeloperoxidase (MPO), and human neutrophil lipocalin (HNL) in COPD patients as compared with controls, and have postulated that the cytokines TNF- $\alpha$  and IL-8 play a role in propagating the inflammatory response in COPD. We have now studied the effects of inhaled and oral glucocorticoids on these inflammatory indices in induced sputum. Initially, we studied the effect of a 2-wk course of inhaled budesonide (800 mg twice daily for 2 wk) in 13 patients with severe COPD (mean FDV<sub>1</sub>: 35% predicted). There was no clinical benefit in either lung function or symptom scores, and no significant change in the inflammatory indices as measured by total and differential cell counts and concentrations of TNF- $\alpha$  eosinophil activation markers ECP and EPO, and neutrophil activation markers MPO and HNL. Because the lack of anti-inflammatory effect might have been due to poor drug delivery as a result of severe airflow limitation, we undertook a study examining the antiinflammatory effect of oral prednisolone (30 mg daily for 2 wk) in patients with COPD and undertook the same measurements in 10 patients with atopic asthma. Sputum eosinophil numbers, ECP, and EPO were significantly reduced in the asthmatic patients but were not modified in COPD. This confirms the clinical impression that inhaled steroids have little antiinflammatory effect, at least in the short term in this group of patients, and suggests that the inflammatory process in COPD is resistant to the antiinflammatory effect of glucocorticoids. **Keatings VM, Jatakanon A, Worsdell YM, Barnes PJ. Effects of inhaled and oral glucocorticoids on inflammatory indices in asthma and COPD.**

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Chronic obstructive pulmonary disease (COPD) is clinically characterized by progressive airflow limitation and intermittent exacerbations, usually precipitated by infection. The main etiologic factor is cigarette smoking, and the cessation of smoking is the only intervention that causes an improvement in the natural history of the condition (1, 2). Long-term domiciliary oxygen therapy prolongs life in subjects with cor pulmonale secondary to COPD, and its beneficial effect on survival is presumably due to effects on the pulmonary circulation (3).

Both asthma and COPD are characterized morphologically by the presence of airway inflammation (4-7). In asthma, there are increased numbers of mast cells and eosinophils, and degranulation of these cells has been shown to occur following allergen challenge. There is increased expression of cytokines interleukin (IL)-2, IL-3, IL-4, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) in asthmatic patients compared with nonasthmatic controls (8). These cytokines contribute to the recruitment and activation of eosinophils into the airways.

Cytokine gene expression is known to be reduced both *in vitro* (9) and *in vivo* (10) in response to corticosteroids, and this has been postulated as the mechanism of action of corticosteroids in this condition (11). In COPD, studies have shown a predominantly neutrophilic infiltrate (12, 13), and it is thought that the consequent protease burden, in addition to reduced antiprotease capacity, is contributory to the development of irreversible airflow obstruction. In addition to the predominantly neutrophilic infiltrate in the airway lumen, a lymphocytic infiltrate has been demonstrated in the airway submucosa and alveolar parenchyma (6, 7, 14). The mechanisms for this inflammatory response are not fully known. Recently, increased expression of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and E-selectin in the endothelium has been demonstrated in bronchial biopsy of patients with COPD (15), and there are increased concentrations of these adhesion molecules in the serum of patients with COPD compared with normal control subjects (16). We have previously described high concentrations of the cytokines IL-8 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in induced sputum from patients with COPD (17), and these cytokines may be involved in the recruitment of neutrophils and upregulation of adhesion molecules on the endothelium (18, 19). If steroids do play a role in the treatment of COPD, it is reasonable to suggest that they act via downregulation of the cytokines and adhesion molecules, with a consequent reduction in cell migration and activation. We have also found high concentrations of the eosinophil activation markers eosinophil cationic protein (ECP) and eosin-

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Correspondence and requests for reprints should be addressed to Prof. P. J. Barnes, Department of Thoracic Medicine, National Heart and Lung Institute, Dovehouse St., London SW3 6LY, UK.

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ophil peroxidase (EPO), and of the neutrophil granule proteins myeloperoxidase (MPO) and human neutrophil lipocalin (HNL) in induced sputum compared with the other subject groups, suggesting that a vigorous inflammatory process is ongoing in the airways of subjects with COPD (20).

Glucocorticoids, both inhaled and oral, are established as effective treatment in asthma, causing improvement in clinical symptoms and spirometry within a short period of time (21, 22). In COPD, however, no such short-term improvement is seen, and the unresponsiveness of FEV<sub>1</sub> is often used as a defining criterion for the exclusion of a diagnosis of asthma (22–24). But the role of glucocorticoids in the management of COPD is controversial (22, 23). Some studies have suggested that long-term oral prednisolone can reduce the deterioration of FEV<sub>1</sub>, and clinical trials of the effect of long-term inhaled steroids are currently underway (24). The findings in clinical studies of corticosteroids in COPD are contradictory. Studies using inhaled steroids have shown a marginal increase in FEV<sub>1</sub> over a 3-wk period in patients receiving high-dose inhaled steroids (25), and other studies have suggested a reduction in long-term decline in FEV<sub>1</sub> in patients taking oral prednisolone at a dose of more than 7.5 mg/d (26, 27). These studies examined patients with a high level of reversibility following a use of bronchodilator, suggesting that many asthmatic subjects must have been included. The clinical effects of a 2-wk course of prednisolone compared with placebo showed that neither skin prick tests, radioallergen sorbent test (RAST), sputum or blood eosinophilia, age, nor bronchodilator response predicted a response to the steroid. One study has shown a selective increase in the protease inhibitor alpha-1 chymotrypsin following 7 d of treatment with prednisolone (28).

If the inflammation in COPD is considered to play a central pathogenic role in the condition, as in asthma, a reduction in the inflammation may accompany a clinical benefit, and if steroid responsiveness is present, it should be apparent within a short period of time. We wished to determine whether the inflammation in COPD is sensitive to the effect of inhaled or oral glucocorticoids, and whether any change is related to clinical benefit. We initially examined the effect of a 2-wk period of treatment with inhaled budesonide (800 µg twice daily) and placebo on both pulmonary function and markers of inflammation in induced sputum in patients with severe COPD. Because there may be problems with inhaled steroids reaching the lower airways in patients with severe airflow obstruction, we then went on to study the effect of oral glucocorticoids (prednisolone 30 mg daily for 2 wk) on the same parameters. In addition, we included patients with asthma as a positive control for the effects of steroids on inflammatory indices in induced sputum.

## METHODS

### Patients

**Budesonide study.** We recruited 15 patients aged 45 to 78 yr (nine males and six females) with stable COPD from the outpatient departments of the Royal Brompton Hospital, St. George's Hospital, and Chelsea and Westminster Hospital, London, and a local general practice. Inclusion criteria for entry were FEV<sub>1</sub>/FVC < 70%, FEV<sub>1</sub> < 70% predicted value, reversibility with inhaled albuterol of < 10% of predicted FEV<sub>1</sub>, and a smoking history of at least 10 pack-yr. Patients with any history of asthma or variability in symptoms, and patients who had taken inhaled or oral steroids or had suffered a respiratory tract infection or exacerbation of their airways disease in the previous 6 wk were excluded.

**Prednisolone study.** Eleven patients with mild atopic asthma were recruited from the outpatient clinic of The Royal Brompton Hospital and from among a panel of volunteers for clinical studies, and nine subjects with COPD were recruited as described earlier. Inclusion criteria for asthmatic patients included nonsmoking subjects with stable asthma with a demonstrated reversibility following 200 µg inhaled albuterol > 15% of initial FEV<sub>1</sub> or > 10% predicted FEV<sub>1</sub>. All of the subjects

were atopic, with positive skin prick testing to at least one of four common aeroallergens (Grass pollen, cat dander, *Dermaphagoides pteronyssinus*, *Aspergillus fumigatus*). Subjects were excluded if they had taken inhaled or oral steroids or had suffered an exacerbation of asthma or respiratory tract infection within the previous 8 wk.

All subjects gave written informed consent, and the studies were approved by the ethics committees of the Royal Brompton Hospital.

### Study Design

Both studies had a sequential single-blind, crossover design. Each study had a 3- to 7-d run-in period during which medications were kept stable, followed by a 2-wk placebo period and a 2-wk treatment period. In the inhaled steroid study the treatment period consisted of budesonide 800 mg twice daily (Astra Draco, Lund, Sweden), and in the prednisolone study, oral prednisolone 30 mg daily. The clinical parts of the studies were single-blind, but all differential cell counting and assays were carried out in a double-blind fashion. Each subject kept a diary card during the study, detailing daily morning peak flow and use of reliever inhaler (in all cases albuterol). Subjects with COPD were also instructed to grade daily shortness of breath score as follows, 0 = none, 1 = mild with no effect on routine activity, 2 = moderate (affecting routine activity), and 3 = severe. Asthmatic subjects kept a daily score of wheeze, 0 = none, 1 = mild with no effect on routine activity, 2 = moderate, affecting routine activity, and 3 = severe.

At the end of the run-in and treatment periods, subjects attended the laboratory. At each visit, spirometric data were recorded at baseline and at 15 min following inhalation of 200 mg albuterol given via metered dose inhaler (MDI) with a spacing device (Volumatic, Allen and Hanburys, Greenford, Middlesex, UK). Diary cards were collected and a sputum induction was done. The sputum sample was analyzed for total and differential cell counts, and assayed for TNF-α, ECP, EPO, MPO, and HNL concentrations.

### Sputum Induction and Processing

Sputum induction was done 15 min after inhalation of 200 µg albuterol via an MDI. The aim of the procedure was explained to the subject, who was instructed to mouthwash thoroughly with water prior to the induction. Subjects inhaled 3.5% saline at room temperature, nebulized via an ultrasonic nebulizer (De Vilbiss 99, De Vilbiss, Heston, UK) at maximum output. Subjects were encouraged to cough deeply at 5 min and at 3-min intervals thereafter. Sputum was collected into two polypropylene pots and saliva was discarded into a bowl. The initial sample of sputum was discarded. Following the sputum induction, spirometry was repeated. If the FEV<sub>1</sub> had fallen, the subject was required to wait until it had returned to baseline value. The sputum samples were kept at 4° C for not more than 2 h prior to further processing.

The volume of the sample was recorded and the sample was diluted with 2 ml of Hanks' balanced salt solution (HBSS) containing 1% dithiothreitol (DTT) (Sigma Chemicals, Poole, UK), and gently vortexed at room temperature. When homogeneous, samples were further diluted with HBSS and again vortexed briefly. They were then spun at 300 × g for 10 min, and the cell pellet was resuspended. The supernatant was decanted, aliquoted, and stored at -70° C for later assay for cytokines and cell activation markers. Total cell counts were done on a hemocytometer using Kimura stain, and slides were made with a cytopsin (Shandon, Runcorn, UK) and stained with May-Grünwald-Giemsa stain for differential cell counts, which were done by an observer blind to the clinical characteristics of the subject.

### Sputum Assays

**TNF-α assay.** TNF-α concentrations were measured with an amplified sandwich-type enzyme-linked immunosorbent assay (ELISA). Ninety-six-well microtiter plates (Greiner Labortechnik Ltd., Dursley, Gloucestershire, UK) were coated with 100 µl of mouse monoclonal anti-TNF-α antibody (Serotec, Oxford, UK), at a 1:400 dilution and left for 2 h at 37° C. Plates were then washed with phosphate-buffered saline (PBS) containing 0.05% vol/vol Tween and immediately treated with bovine serum albumin (BSA) 5% vol/vol for 30 min at 37° C. After further washing, TNF-α standards, quality controls, and samples were added to the plates and left for 18 h at 4° C. The plates were washed and incubated for 2 h at room temperature with 100 µl of rabbit anti-human TNF-α polyclonal antibody, washed again, and incubated for a further

TABLE 1  
LUNG FUNCTION IN PATIENTS WITH COPD AFTER  
PLACEBO AND BUDESONIDE TREATMENT

Patient No.	FEV <sub>1</sub>		FEV <sub>1</sub> pb		FVC		PEFR	
	Placebo	Budes.	Placebo	Budes.	Placebo	Budes.	Placebo	Budes.
1	0.78	0.57	0.77	0.73	1.98	1.98	ur	ur
2	0.68	0.65	0.57	0.73	1.69	1.8	ur	ur
3	0.64	0.65	0.76	0.76	1.45	1.49	ur	ur
4	0.59	0.69	0.83	0.78	0.98	1.42	ur	ur
5	2.63	2.37	2.94	2.95	4.48	4.35	436	464
6	0.58	0.62	0.76	0.75	1.32	1.44	ur	ur
7	1.63	1.58	1.68	1.74	3.04	2.35	253	223
8	0.79	0.98	0.77	0.98	2.16	1.98	191	181
9	0.84	0.9	0.89	0.9	2.28	2.1	234	235
10	2.05	2.05	2.25	2.2	2.73	2.8	435	461
11	0.87	0.9	1.05	1.27	2.28	2.45	139	128
12	0.91	0.96	0.88	1.02	4.14	4.2	172	167
13	0.47	0.4	0.48	0.52	1.04	1.2	146	120
Mean	1.04	1.02	1.13	1.18	2.34	2.30	250	247
SEM	0.19	0.17	0.21	0.20	0.32	0.30	45.6	52.4

Definition of abbreviations: FEV<sub>1</sub> = forced expiratory volume in first second; FEV<sub>1</sub> pb = FEV<sub>1</sub> following inhaled albuterol 200 µg; FVC = forced vital capacity; PEFR = mean morning peak expiratory flow rate during treatment; Budes. = budesonide; ur = unrecordable peak flow rate.

Data are for 13 patients with COPD.

h at room temperature with an alkaline phosphatase-conjugated donkey anti-rabbit polyclonal IgG antibody (diluted 1:2,000). Excess antibody was again washed off and plates were developed with a p-nitrophenyl phosphate assay kit (No. 50-80-00; KPL/Dynatech Laboratories Ltd., Billingham, Sussex, UK). The optical density of the wells was read using a plate photometer. The detection limit of the assay is 470 pmol.

**IL-8 assay.** IL-8 concentrations were measured with a competitive radioimmunoassay (RIA) (29). Human recombinant IL-8 was radiolabeled with <sup>125</sup>I. Samples were mixed with an equal volume of 22% polyethylene glycol/1% protamine sulfate, incubated for 1 h at 4° C, and centrifuged at 5,420 × g for 10 min at 4° C. The resulting supernatant fluid (100 µl) was mixed with 50 µl [<sup>125</sup>I]-human IL-8 (0.5 ng). After 24 h incubation at room temperature, 50 µl of donkey anti-goat IgG antibody (1:30 dilution) was added and incubated for 16 h at room temperature. After addition of 1 ml PBS containing 0.1% sodium azide and immediate centrifugation, the supernatant fluid was removed by suction and antibody-bound radioactivity was counted in a gamma counter. All samples were assayed in duplicate with human recombinant IL-8 standards. The lower limit of detection was 197 pmol IL-8, and non-specific binding was 5.0%.

**ECP assay.** ECP concentrations were measured with a commercially available RIA, a generous gift from Pharmacia Diagnostics AB, Uppsala, Sweden.

**EPO assay.** Anti-EPO monoclonal antibody was coupled to ImmunoCAP (Pharmacia Diagnostics). Samples and standards were added and incubated for 30 min at room temperature. After washing, anti-EPO labeled with β-galactosidase was added. Following incubation for 2.5 h at room temperature, suspensions were washed and the substrate 4-methylumbelliferyl-β-D-galactoside was added. Fluorescence was measured after 10 min, a standard curve was constructed, and EPO concentrations were determined. Cross-reactivity with ECP was < 0.3% and with MPO < 0.01%.

**MPO assay.** MPO concentrations were determined with a commercially available RIA (Pharmacia Diagnostics) according to the manufacturer's instructions. Cross-reactivity with ECP and MPO was < 0.1%.

**HNL assay.** Monoclonal antibodies (mAb) were raised in rats immunized with HNL. One mAb was coupled to ImmunoCAP (Pharmacia Diagnostics). Samples and standards were added and incubated for 30 min at room temperature. After washing, β-galactosidase-labeled anti-HNL was added. Following incubation for 2.5 h at room temperature, suspensions were washed and 4-methylumbelliferyl-β-D-galactoside was added. Fluorescence was measured and HNL concentrations were determined.

#### Statistical Analysis

Data are expressed as the mean ± SEM. Statistical analysis of comparisons

between groups was performed with Student's *t* test for parametric data and with Wilcoxon's rank sum test for nonparametric data. Two-tailed tests were performed, and a *p* value of < 0.05 was considered significant.

## RESULTS

### Budesonide Study

**Clinical parameters.** Patients had severe airflow limitation, with a mean FEV<sub>1</sub> of 35.1 ± 1.3% predicted. All subjects were non-atopic, with negative results on skin prick testing to four common aeroallergens. Mean cigarette smoking history was 48 ± 2 pack-yr, and six subjects were current smokers. Two subjects withdrew from the study (one due to work commitments and another who did not wish to have a repeat sputum induction). All subjects produced an adequate specimen of sputum. Mean FEV<sub>1</sub> was 0.93 ± 0.18 L at baseline, 1.04 ± 0.19 L with placebo, and 1.02 ± 0.17 L with budesonide (i.e., no difference between placebo and active treatment periods). Post-albuterol FEV<sub>1</sub> was 1.09 ± 0.27 L, 1.13 ± 0.21 L, and 1.18 ± 0.20 L at baseline, on placebo, and active treatment periods, respectively (Table 1).

Eight of the 13 patients had recordable peak flows. Mean peak flow in these patients was 250 ± 45.6 L/min in the placebo period and 247 ± 52.4 L/min during treatment with budesonide.

Use of reliever medication was 2.3 ± 0.3 puffs/d on placebo and 2.13 ± 0.4 on budesonide, (*p* = NS). Shortness-of-breath scores were 1.18 ± 0.3 on placebo and 1.34 ± 0.4 on budesonide (*p* = NS).

**Inflammatory indices.** Total and differential cell counts did not change from baseline on placebo or active treatment (Table 2). The eosinophil number was skewed by one patient (Subject 3) whose eosinophil count was initially high (9.6%) and fell to zero following budesonide.

There was no significant change in ECP, EPO, MPO, or HNL after placebo or budesonide treatment periods. Similarly, the concentration of TNF-α remained unchanged throughout the study.

### Prednisolone Study

One asthmatic subject withdrew from the study during the placebo limb due to work commitments, and one subject with COPD withdrew due to failure to fully understand how to fill in the diary card. Results therefore relate to 10 asthmatic sub-



TABLE 2

INFLAMMATORY INDICES IN INDUCED SPUTUM IN COPD AFTER TREATMENT WITH BUDESONIDE AND PLACEBO

	Baseline	Placebo	Budesonide	p Value
Total cell count/ml	6.3 ± 2.0	5.7 ± 2.0	4.8 ± 1.9	NS
Macrophages, %	28.5 ± 6.0	27.6 ± 3.7	27.5 ± 5.1	NS
Neutrophils, %	67.9 ± 6.3	69.9 ± 4.5	69.9 ± 5.1	NS
Eosinophils, %	2.7 ± 1.2	2.0 ± 1.1	1.0 ± 0.4	NS
Lymphocytes, %	0.9 ± 0.6	1.4 ± 0.1	1.6 ± 0.3	NS
TNF-α pg/ml	760.2 ± 119	463.5 ± 35.7	715.5 ± 90.8	NS
IL-8 nm	3.3 ± 1.6	3.5 ± 2.0	2.1 ± 1.1	NS
ECP μg/L	835.8 ± 0.40	730.3 ± 0.29	910.5 ± 0.21	NS
EPO μg/L	262.4 ± 53.2	85.4 ± 15.1	41.9 ± 5.1	NS
MPO mg/L	8.05 ± 1.49	4.17 ± 0.58	2.97 ± 0.39	NS
HNL mg/L	10.48 ± 1.02	8.98 ± 0.67	8.47 ± 0.68	NS

Definition of abbreviations: TNF-α = tumor necrosis factor-α; IL-8 = interleukin-8; ECP = eosinophil cationic protein; EPO = eosinophil peroxidase; MPO = myeloperoxidase; HNL = human neutrophil lipocalin.

Mean ± SEM values of 13 patients are shown.

jects and eight subjects with COPD. The mean age of the asthmatic patients was 29.8 ± 3.4 yr and their mean FEV<sub>1</sub> was 95.9 ± 5.7% predicted. The patients with COPD had a mean age 64.1 ± 5.1 yr and FEV<sub>1</sub> of 48.0 ± 6.8% predicted.

**Clinical parameters.** Lung function and diary card scores for both patient groups are shown in Table 3. There was a significant increase in FEV<sub>1</sub> and morning peak flow following prednisolone treatment in asthmatic patients, and the use of reliever inhaler was reduced significantly from 1.2 puffs/d during the placebo period to 0.5 puffs/d during treatment with prednisolone ( $p < 0.05$ ). In contrast, there were no significant changes in lung function, peak flow, or shortness-of-breath score in the subjects with COPD. There was no difference in use of reliever

inhaler with placebo and prednisolone treatment (2.9 ± 0.4 and 2.8 ± 0.3 puffs/d with placebo and prednisolone, respectively).

**Differential cell counts.** Differential cell counts are shown in Tables 4 and 5. Neutrophil counts were significantly higher in the COPD patients (mean 61.56 ± 2.5%, compared with 17.95 ± 1.20% in the asthma patients at baseline. Eosinophils were significantly higher in the asthma patients, at 6.66% ± 0.98%, than in the patients with COPD, at 0.58 ± 0.11%. ( $p < 0.0001$ ). Changes in eosinophil numbers with treatment are shown in Figure 1. In the asthma patients there was a statistically significant decrease in eosinophils following treatment with prednisolone as compared with placebo, whereas in the COPD patients eosinophil numbers did not change after either the treatment or placebo period. In both groups of patients, numbers of other cell types were not altered following treatment with prednisolone.

**Markers of cell activation.** ECP concentrations were similarly elevated in both subject groups. In subjects with asthma, there was a significant decrease in ECP concentrations after prednisolone ( $p < 0.05$ ), with no effect after placebo. In the patients with COPD, however, the concentration of ECP did not change following either treatment. Similarly, there was a significant decrease in EPO after steroid treatment in the asthma patients but this was not significant in COPD (Figure 2).

Although there was a decrease in concentrations of MPO and HNL in both patient groups after prednisolone, this did not reach significance (Tables 4 and 5).

There was no change in TNF-α concentrations in either the asthmatic or the COPD group of subjects following treatment with prednisolone as compared with placebo (Tables 4 and 5).

## DISCUSSION

Our study has confirmed the irreversibility of chronic airflow limitation and lack of response to short-term steroid therapy. In

TABLE 3  
SPIROMETRIC VALUES FOR PATIENTS WITH ASTHMA AND COPD FOLLOWING PLACEBO AND PREDNISOLONE

Patient No.	FEV <sub>1</sub>		FEV <sub>1</sub> pb		FVC		PEFR	
	Placebo	Pred.	Placebo	Pred.	Placebo	Pred.	Placebo	Pred.
<b>Asthma</b>								
1	4.2	4.8	4.77	4.95	5.45	5.5	623	634
2	3.31	3.41	3.57	3.61	3.97	3.96	490	500
3	4.7	5.11	4.88	5.18	5.75	5.95	590	663
4	2.66	2.45	2.95	2.86	3.42	3.5	353	364
5	4.62	4.94	4.9	5.2	5.73	5.26	644	660
6	3.46	3.5	3.89	3.92	4.16	4.17	493	582
7	2.86	3.12	3.18	3.55	3.93	4.35	398	510
8	5.61	5.69	5.75	5.8	6.42	6.5	729	724
9	3.38	3.4	3.65	3.99	4.76	4.7	501	525
10	2.73	3.13	2.65	3.29	3.43	3.52	396	408
Mean	3.75	3.96*	4.02	4.24*	4.702	4.741	521	557*
SEM	0.32	0.32	0.34	0.41	0.36	0.34	41	39
<b>COPD</b>								
11	0.72	0.71	0.75	0.95	1.63	2.1	232	234
12	0.6	0.46	0.46	0.31	1.95	2.17	ur	ur
13	1.88	2	2.10	2.19	2.62	2.8	443	453
14	0.84	0.59	0.73	0.77	2.98	3.4	146	146
15	0.98	0.9	1.02	0.98	3.2	3.88	205	197
16	0.98	0.92	1.11	0.92	1.98	1.84	213	226
17	1.79	2.5	1.65	2.44	2.25	3.05	450	478
18	2.38	2.28	2.65	2.57	3.65	3.4	389	373
Mean	1.27	1.30	1.31	1.39	2.53	2.83	297.2	301.5
SEM	0.25	0.32	0.29	0.33	0.27	0.28	48.5	50.8

Definition of abbreviations: FEV<sub>1</sub> = forced expiratory volume in first second; FEV<sub>1</sub> pb = FEV<sub>1</sub> following inhaled albuterol 200 μg; ur = unrecordable peak flow rate; Pred. = prednisolone; Data are for 10 patients with asthma and eight patients with COPD.

\*  $p < 0.05$  compared with placebo value.

TABLE 4  
TOTAL AND DIFFERENTIAL CELL COUNTS, CELL ACTIVATION MARKERS,  
AND CONCENTRATIONS OF TNF- $\alpha$  IN ASTHMATIC SUBJECTS AT BASELINE AND  
AT END OF TREATMENT PERIODS (PREDNISOLONE STUDY)

	Baseline	Placebo	Prednisolone	p Value
Total cell count/ml	1.48 $\pm$ 0.12	1.34 $\pm$ 0.12	0.81 $\pm$ 0.07	NS
Macrophages, %	74.11 $\pm$ 1.68	60.15 $\pm$ 2.16	67.19 $\pm$ 1.77	NS
Neutrophils, %	17.95 $\pm$ 1.20	31.63 $\pm$ 1.68	30.89 $\pm$ 1.68	NS
Eosinophils, %	6.66 $\pm$ 0.98	7.28 $\pm$ 1.04	0.99 $\pm$ 0.25	p < 0.05
Lymphocytes, %	0.42 $\pm$ 0.04	0.17 $\pm$ 0.04	0.15 $\pm$ 0.04	NS
TNF- $\alpha$ , pg/ml	1,743 $\pm$ 193.6	1,760 $\pm$ 228	2,049 $\pm$ 246	NS
ECP, $\mu$ g/L	687.0 $\pm$ 87.1	871.6 $\pm$ 121.8	80.2 $\pm$ 14.2	p < 0.05
EPO, $\mu$ g/L	59.2 $\pm$ 6.2	86.4 $\pm$ 5.5	16.4 $\pm$ 3.2	p < 0.05
MPO, mg/L	0.6 $\pm$ 0.1	1.0 $\pm$ 0.1	0.2 $\pm$ 0.05	NS
HNL, mg/L	3.4 $\pm$ 0.3	1.7 $\pm$ 0.1	1.8 $\pm$ 0.2	NS

Definition of abbreviations: TNF- $\alpha$  = tumor necrosis factor- $\alpha$ ; ECP = eosinophil cationic protein; EPO = eosinophil peroxidase; MPO = myeloperoxidase; HNL = human neutrophil lipocalin.

the patients with COPD, there were no significant improvements in any of the clinical parameters studied. Although the study could not be fully double-blind for logistical reasons, the cell counts and protein assays were all done in a double-blind fashion. The previous demonstration of a neutrophil influx and high concentrations of TNF- $\alpha$  and cell activation markers in the airways indicates an active inflammatory process in COPD, suggesting that steroids may be beneficial. TNF- $\alpha$  has been implicated in the pathogenesis of asthma, and we have previously found high concentrations in COPD, suggesting that this cytokine is also involved in the inflammation in COPD. Although steroids do not change the airway function and symptoms in these patients, it is possible that an effect on inflammatory indices may be observed and that this could have long-term clinical effects. It has previously been shown that ECP concentrations in asthma are reduced following a 2-wk treatment period with prednisolone (30). This study confirms these findings and shows that another marker of eosinophil activation, EPO, can be reduced by treatment with steroids. It has been suggested that steroids work in asthma through reduction of cytokine gene expression, thus reducing the cytokine effects on adhesion molecule expression and chemotaxis, resulting in a reduced inflammatory influx. This study shows no effect of prednisolone on levels of TNF- $\alpha$  in asthma, despite *in vitro* evidence that TNF- $\alpha$  production from monocytes is reduced by dexamethasone and budesonide (31). Similarly, IL-8 was not inhibited after inhaled glucocorticoids in patients with COPD, despite the fact that glucocorticoids inhibit IL-8 transcription in human airway epithelial cells *in vitro*

(32). TNF- $\alpha$  and IL-8 may be less sensitive to inhibition by steroids than other cytokines such as regulated on activation, normal T cell expressed and secreted (RANTES) and IL-5, which are thought to play an important part in eosinophil chemotaxis in asthma. Eosinophil survival is also reduced following treatment as a result of increased eosinophil apoptosis, probably due to inhibitory effects of glucocorticoids on growth factors such as IL-5 and GM-CSF (33). Because there is no evidence that glucocorticoids actually reduce degranulation of eosinophils, the reduction in granule proteins is secondary to reduced numbers of eosinophils rather than to reduced production of ECP and EPO by the cell. The lack of reduction of neutrophil numbers and markers clearly demonstrates a difference in steroid effects on eosinophils and neutrophils. Steroids are known to cause a circulating neutrophilia, and it has been shown that neutrophil apoptosis is reduced *in vitro* by treatment with glucocorticoids (34), thus prolonging the survival of neutrophils in tissues and reducing their clearance by macrophages. The initial study with inhaled glucocorticoids gives the impression that inhaled steroids are of little value in modifying inflammation in COPD, but because these patients had severe airflow limitation, we considered that the lack of effect might have been due to poor deposition of the drug in the airways. The second study, using prednisolone as the active treatment, was therefore undertaken and a group of asthmatic patients recruited as a positive control to demonstrate that our method of sputum induction was a sensitive tool with which to detect the effect of intervention.

ECP and EPO concentrations were high in the patients with

TABLE 5  
TOTAL AND DIFFERENTIAL CELL COUNTS, CELL ACTIVATION MARKERS  
AND CONCENTRATIONS OF TNF- $\alpha$  IN PATIENTS WITH COPD AT BASELINE  
AND AT END OF TREATMENT PERIODS (PREDNISOLONE STUDY)

	Baseline	Placebo	Pred.	p Value
Total cell count/ml	5.62 $\pm$ 1.32	5.25 $\pm$ 0.78	5.78 $\pm$ 0.88	NS
Macrophages, %	37.76 $\pm$ 2.49	34.70 $\pm$ 1.74	29.50 $\pm$ 3.47	NS
Neutrophils, %	61.56 $\pm$ 2.5	65.0 $\pm$ 1.73	70.21 $\pm$ 3.44	NS
Eosinophils, %	0.58 $\pm$ 0.11	0.68 $\pm$ 0.10	0.20 $\pm$ 0.02	NS
Lymphocytes, %	0.1 $\pm$ 0.02	0.25 $\pm$ 0.06	0.03 $\pm$ 0.01	NS
TNF- $\alpha$ , pg/ml	550 $\pm$ 20	496 $\pm$ 36	624 $\pm$ 47	NS
ECP, $\mu$ g/L	575.6 $\pm$ 87.6	677.2 $\pm$ 202.2	989.2 $\pm$ 234.8	NS
EPO, $\mu$ g/L	70.0 $\pm$ 13.7	33.1 $\pm$ 5.8	90.0 $\pm$ 2.5	NS
MPO, mg/L	10.3 $\pm$ 3.1	8.6 $\pm$ 2.1	5.0 $\pm$ 1.2	NS
HNL, mg/L	17.8 $\pm$ 3.9	11.7 $\pm$ 1.9	11.5 $\pm$ 1.4	NS

Definition of abbreviations: TNF- $\alpha$  = tumor necrosis factor- $\alpha$ ; ECP = eosinophil cationic protein; EPO = eosinophil peroxidase; MPO = myeloperoxidase; HNL = human neutrophil lipocalin; Pred. = prednisolone.

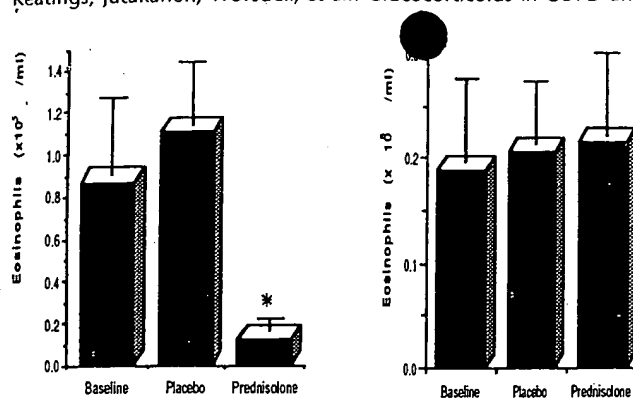


Figure 1. Changes in eosinophil numbers following treatment periods in asthmatic patients (left panel) and subjects with COPD (right panel). \* $p < 0.05$ .

COPD, and correlated with eosinophil numbers, suggesting that the proteins are derived from highly activated eosinophils. In contrast to asthma, however, concentrations of ECP and EPO were unchanged following treatment with prednisolone. Given that glucocorticoids increase neutrophil survival, it is not surprising they do little to modify inflammation in COPD, but the lack of inhibition of eosinophil activation by prednisolone in COPD is not readily explained. It does suggest that there is at least one steroid-unresponsive step in the development of this process that is different from the eosinophil activation process in asthma. This may in part explain the relative resistance of COPD to treatment with steroids.

Sputum induction was used in this study because it was a

method of ensuring that subjects were able to produce a sample at each visit since not all subjects with COPD can produce sputum on demand. Asking subjects to bring a morning sample of sputum to the visit would lengthen the period of time between production and processing, which could result in samples containing variable numbers of degenerate cells.

It is possible that a 14-d treatment period was too short to detect a change in inflammatory indices, but in the asthma patients, a clinical benefit and an antiinflammatory effect was seen within this period, and a 2-wk period is commonly used in clinical practice when a trial of steroids is administered to patients with COPD. Two weeks of oral corticosteroid therapy have been shown to modify ECP concentrations in asthma. We are continuing to study the effect of corticosteroids in COPD, using a longer treatment period and a more potent inhaled steroid. In summary, we have demonstrated that in patients with COPD, the intense inflammatory process is also unresponsive to therapy with either a 2-wk course of high-dose inhaled steroids or of high-dose oral prednisolone. This confirms the clinical impression that these drugs are of little value in COPD, at least in the short term. Since it is clear that the inflammatory mechanisms in COPD differ significantly from those in asthma, further study should be directed at the elucidation of these mechanisms with a view to discovering more specific therapy for this condition.

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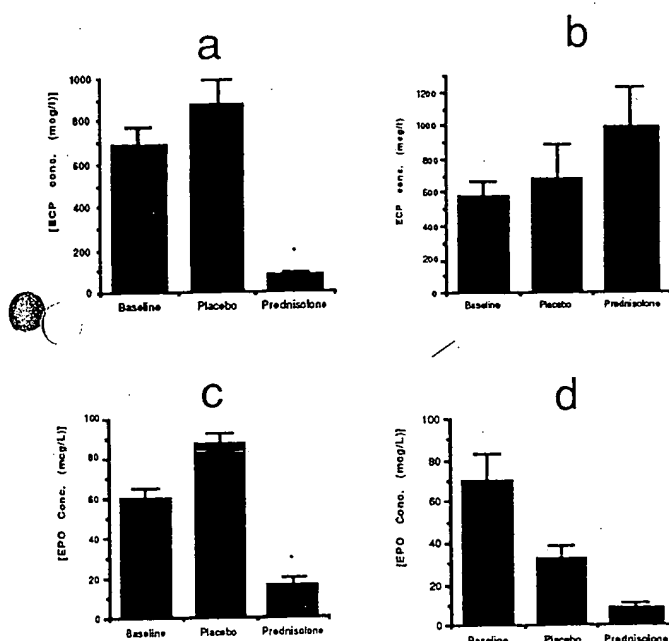


Figure 2. Changes in concentration of eosinophil cationic protein (ECP) in patients with asthma (a) and COPD (b), and in eosinophil peroxidase (EPO) in patients with asthma (c) and COPD (d) before and after treatment with oral prednisolone or placebo. Mean values  $\pm$  SEM of 10 asthma and eight COPD patients are shown: \* $p < 0.05$  for difference between two treatment groups.

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# Failure of Inhaled Corticosteroids to Modify Bronchoconstrictor or Bronchodilator Responsiveness in Middle-Aged Smokers with Mild Airflow Obstruction\*

Ann Watson, H.N.D.; Tow Keang Lim, M.B., M.Med.; Helen Joyce, S.R.N.; and Neil B. Pride, M.D.

We have compared the effects of three-month periods of treatment with an inhaled corticosteroid, budesonide 600 µg twice daily and with placebo on bronchial responses to inhaled histamine and to bronchodilators in a double-blind crossover trial in 14 middle-aged male smokers (mean age, 59.6 years) with mild airways obstruction (mean FEV<sub>1</sub> 2.42 L, 80 percent predicted [range, 48 to 110 percent]). Responsiveness to inhaled histamine was assessed monthly by the provocative concentration (mg/ml) reducing FEV<sub>1</sub> by 20 percent (PC<sub>20</sub>). Bronchodilator response to a combination of inhaled salbutamol (5 mg) and ipratropium (0.5 mg) was assessed before and after three months' treatment. Compliance with treatment was checked by weighing aerosol canisters, and by measuring plasma budesonide and metabolites. There was no significant change in FEV<sub>1</sub> (budesonide mean 2.38 L [SEM 0.17] vs placebo 2.40 L [0.17]), vital capacity (budesonide mean 3.69 L [0.17] vs placebo 3.81 L [0.17]) or in bronchodilator responsiveness (mean increase over baseline FEV<sub>1</sub>, budesonide 11.6 [2.7] percent vs placebo 10.5 [3.2] percent). There was a small overall reduction in bronchoconstrictor responsiveness over the period of the trial, but there was no effect of 12 weeks

of budesonide treatment compared with 12 weeks of placebo treatment (mean log PC<sub>20</sub> during budesonide 0.595 [SEM 0.063], placebo 0.591 [SEM 0.055]). Following the three-month crossover trial, six men continued for nine more months to receive budesonide in a single-blind trial and the results were compared with those in six men who took no active treatment for the subsequent nine months. No improvements in baseline spirometry, home peak flow measurements, bronchoconstrictor or bronchodilator responsiveness were observed after 12 months of budesonide treatment. Thus, a regimen of budesonide treatment that consistently attenuates bronchial responsiveness in asthmatic subjects had no effect in these men; larger at-home trials will be required to establish whether a subgroup of smokers shows a favorable response.

(Chest 1992; 101:350-55)

BHR = bronchial hyperresponsiveness; F<sub>50</sub>CO = fractional concentration of mixed expired carbon monoxide; PC<sub>20</sub> = provocative concentration of inhaled histamine reducing FEV<sub>1</sub> by 20 percent

In subjects with asthma, inhaled corticosteroids consistently attenuate nonspecific bronchial hyperresponsiveness (BHR) to inhaled bronchoconstrictor drugs such as histamine or methacholine.<sup>1-11</sup> In contrast, there is much less information on the effects of inhaled corticosteroids on BHR in nonasthmatic smokers even though corticosteroids are frequently used empirically in the treatment of smokers with chronic obstructive pulmonary disease (COPD). Two recent studies have failed to show any change in BHR associated with this treatment in patients with COPD.<sup>12,13</sup>

We have performed a double-blind crossover trial of the effects of inhaled corticosteroids on (1) bronchial responsiveness to inhaled histamine and to inhaled bronchodilators and (2) baseline spirometry and peak flow measurements in 14 middle-aged male smokers with BHR and mild-to-moderate airflow obstruction. After the double-blind trial over six months, 12 of the men continued for an additional nine months in an

open trial of budesonide or placebo to obtain pilot information on longer-time effects. Preliminary result of this trial have been reported.<sup>14</sup>

## MATERIALS AND METHODS

### Subjects

Fifteen middle-aged male smokers with measurable BHR to inhaled histamine without overt asthmatic features were recruited from those attending a long-term follow-up study of the effects of smoking started in 1974;<sup>15</sup> their rate of change in FEV<sub>1</sub> over the preceding 12 years was therefore known. At initial recruitment and at subsequent follow-up, all men were encouraged to give up smoking. These men were found to have BHR to inhaled histamine in 1982.<sup>16</sup> In that study the majority of smokers, exsmokers, and never-smokers were not responsive to the doses of histamine used. Men with episodic wheezing attacks occurring at rest, or at night, or men who had been receiving bronchodilator treatment, or men who had ever received the diagnosis of asthma were excluded from the study; because positive skin tests to common aeroallergens as found in about a third of the general population, these were not used to exclude subjects. Mean carbon monoxide transfer coefficient was slightly reduced at 1.27 (SEM, 0.06; range, 1.0 to 1.7 mmol·min<sup>-1</sup>·kPa<sup>-1</sup>·l<sup>-1</sup>). Skin reactivity to extracts from nine commonly inhaled antigens (grass pollen, cat and dog dander, mixed feathers, *Alternaria* sp, *Cladosporium* sp, *Aspergillus fumigatus* house dust, and *Dermatophagoides pteronyssinus*) was assessed in

\*From the Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London, England.  
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Table 1—Summary of Data

	Age, yr	Height, m	Current Smoking, cigs/day	Pack years	FEV <sub>1</sub>		FEV <sub>1</sub>		1974-1986 $\Delta$ FEV <sub>1</sub> /Hr <sup>2</sup> , ml/yr/m <sup>3</sup>	PC20 Histamine, mg/ml	Positive Skin Tests	Treatment
					L	%pred	Post bd	%Response				
1	52	1.71	30	57	2.34	72	2.94	26	24.0	0.8	House dust mite	...
2	58	1.79	18	34	2.54	75	2.87	13	15.3	4.5	Nil	...
3	52	1.75	40	66	3.43	101	3.43	0	7.1	5.2	Nil	...
4	66	1.67	20	45	2.92	110	3.12	7	8.6	4.4	Feathers	...
5	63	1.63	30	64	2.22	86	2.49	12	21.5	0.9	Nil	Atenolol
6	57	1.73	30	55	1.51	48	1.75	16	20.9	2.0	Nil	...
7	65	1.72	20	49	1.59	55	2.04	28	10.0	0.7	Nil	Atenolol
8	59	1.72	45	94	2.04	66	2.32	14	21.2	0.9	Nil	Atenolol
9	55	1.68	30	49	2.12	70	2.42	14	17.3	1.6	Feathers, house dust mite, cat, dog	Labetalol
10	57	1.85	12	22	2.30	63	2.40	4	13.1	2.0	Nil	...
11	61	1.80	12	25	3.09	93	3.06	-1	11.7	4.7	Nil	Atenolol
12	66	1.63	20	44	2.20	88	2.34	6	13.4	7.6	Nil	...
13	60	1.73	19	38	2.71	89	2.78	3	9.5	8.5	Nil	...
14	65	1.71	14	32	2.93	103	3.25	11	9.8	14.5	Nil	...
Mean	59.7	1.72	24.3	48.1	2.42	80	2.66	10.9	14.53	2.83*		
(SEM)	(1.3)	(0.17)	(2.7)	(5.0)	(0.15)	(5.0)	(0.13)	(2.3)	(1.49)			

\*Geometric mean.

prick tests in forearm skin. The response to the test was recorded as positive when the mean wheal diameter was more than 2 mm. Because the trial was to extend over the period January to July, men with nasal sensitivity to pollen (as judged by history or positive skin tests) were excluded.

After the first month, one man who was not complying with treatment withdrew due to lack of time to attend the laboratory. The remaining 14 men completed the six-month trial. Their age, smoking habit, baseline spirometry, bronchial responsiveness, preceding decline in spirometry, skin test results, and drug treatment are summarized in Table 1. Five men were being treated with cardioselective  $\beta$ -blocking drugs for mild hypertension that were continued in unchanged dosage throughout the trial. None was receiving bronchodilator drugs or other treatment. Their mean height-corrected decline in FEV<sub>1</sub> over the 12 years preceding the trial was considerably accelerated, and was three times that found in a group of never-smokers followed concurrently.<sup>17</sup>

#### Measurements

At recruitment for this trial, a detailed questionnaire was applied to establish respiratory symptoms, smoking habits, and any history of asthma or allergic disease. At subsequent visits, a separate questionnaire was applied to detect any change in symptoms, smoking habits, treatment, or any recent upper or lower respiratory tract infection or side effects. At each visit fractional concentration of mixed expired carbon monoxide (FeCO) was measured during a period of quiet breathing to corroborate stated smoking habit. No subjects were studied within six weeks of a significant upper or lower respiratory tract infection. If an infection developed, measurements were postponed appropriately and the treatment arm extended.

Forced expiratory volume in 1 s (FEV<sub>1</sub>) was measured in the standing position with a dry bellows spirometer that was calibrated daily. The highest FEV<sub>1</sub> from three technically satisfactory forced expirations expressed at BTPS was taken as the baseline and compared with reference values.<sup>18</sup> The provocative concentration of inhaled histamine reducing FEV<sub>1</sub> by 20 percent (PC20) was then determined with the same equipment and technique as was used in the 1982 study.<sup>18</sup> Subjects wore a noseclip and inhaled a 0.9 percent saline solution followed by doubling concentrations of histamine diphosphate (0.5 to 32 mg/ml) generated by a compressed air-driven nebulizer (Wright) at a flow rate of 7.5 L/min through a

mouthpiece during tidal breathing for 2 min. The output of the nebulizer, which was checked regularly, was 0.14 ml/min. FEV<sub>1</sub> was recorded at 60, 90, and 180 s. In occasional tests where the value at 180 s was lower than at 90 s, another measurement was made at 5 min to be sure the lowest value was obtained after each inhalation. The challenge was terminated when FEV<sub>1</sub> fell below 20 percent of the lowest post-saline solution value or the 32 mg/ml concentration of histamine was reached. The PC20 histamine was determined by linear interpolation from a log dose-response curve.

After recovery from the effects of histamine, the bronchodilator response was assessed. A solution containing 5 mg of salbutamol and 0.5 mg of ipratropium bromide in isotonic solution was nebulized and inhaled via a mouthpiece and the change in FEV<sub>1</sub> was measured 60 min later.

During the last four weeks of each treatment period, morning and evening peak flow measurements were made at home using a peak flow gauge (Wright).

Venous blood was drawn for total white blood cell (WBC) counts, eosinophil count, and total IgE levels at recruitment and at the end of the 12-week treatment periods with budesonide or placebo. Blood budesonide levels were also checked by a radioimmunoassay technique at the end of each treatment period.

#### Treatment

Identical canisters with extension tubes which contained either budesonide (200  $\mu$ g/puff) or placebo were used at a dosage of three puffs twice a day. The dosage of budesonide (600  $\mu$ g twice daily) was judged to be the largest dose that could be given without producing significant hypothalamic-pituitary-adrenal suppression. Compliance with treatment was checked by weighing canisters at each visit and after 12 weeks of each treatment by measuring plasma budesonide levels in peripheral venous blood.

#### Design and Analysis of Double-Blind Crossover Trial

After initial measurements, the men started on a 12-week period of treatment with four-weekly laboratory visits. For the last four weeks (weeks 9 to 12) of each treatment, twice daily peak expiratory flow measurements were made at home. All PC20 values were logarithmically transformed before any calculations. The double-blind, two-period, crossover trial was analyzed for a quantitative response and for interactions between treatment and period as set out in the full description of Hills and Armitage.<sup>19</sup>

### Single-Blind Follow-up

At the last visit of the six-month crossover trial, the men were instructed to continue with their current treatment. The code was subsequently broken and the men continued treatment with either budesonide or placebo in a single-blind trial for the subsequent nine months with three further sets of measurements of baseline FEV<sub>1</sub>, bronchodilator and bronchoconstrictor responsiveness at approximately every three months, and four more weeks of home peak flow measurements in the last four weeks of treatment. One man died of myocardial infarction just after the end of the six-month crossover trial and one man moved out of the London area so that 12 men (six receiving placebo and six receiving budesonide) completed the full period of 12 months with the second treatment. All treatment was then stopped.

The studies were approved by the local Medical School Research Ethics Committee.

## RESULTS

### Double-Blind crossover Trial

**Smoking Habits:** These were generally similar through the trial (as assessed by stated habits and FE<sub>CO</sub>); one patient unsuccessfully tried to quit smoking for a few days during the trial.

**Compliance with Treatment:** In the 14 men who completed the trial, canister weights showed a reduction averaging 0.37 g/day with most individuals showing similar values; qualitative measurements of plasma budesonide also confirmed the presence of budesonide in all the men at the end of the appropriate 12-week period. One subject appeared to be using insufficient aerosol during weeks 9 to 12 of placebo treatment. One man consistently appeared to use less aerosol (about 60 percent of mean value) both during placebo and budesonide treatment.

**Untoward Events:** One man dropped out after four weeks when it became clear his compliance with treatment was poor. Of the remaining 14 men, only one complained of a side effect related to budesonide (hoarseness, throat irritation, and sore tongue); this led to a short break in treatment and postponement of final assessment.

Five men had respiratory tract infections during the trial, although none was very severe; four occurred

during the first or second month of budesonide treatment, one during the third month of receiving placebo. In two men, the total period receiving budesonide was extended by one to two weeks because of these infections.

One of the 14 men died of an acute myocardial infarction a few days after completing the trial; he had been receiving placebo aerosol during the last weeks of the trial, and was the individual who had been poorly compliant with treatment in the first month.

**Changes in Symptoms:** Apart from transient increases in cough and phlegm (and in two of the men associated mild breathlessness) at the time of respiratory infections, no consistent changes in symptoms were noted.

**Changes in Venous Blood:** The lowest mean value of eosinophil counts was found at the end of the 12-week period with budesonide (Table 2). There were no significant changes in mean venous blood total WBC counts or IgE.

**Changes in Baseline Spirometry and Bronchodilator Responsiveness (Table 3):** Mean values of FEV<sub>1</sub>, vital capacity (VC), and laboratory measurements of peak expiratory flow (PEF) showed very little variation through the trial. The mean difference between highest and lowest values in the seven baseline measurements of FEV<sub>1</sub> was 0.27 L; the largest differences were 0.44 and 0.55 L. There was no effect of period or drug or drug/period interaction on prebronchodilator values of FEV<sub>1</sub> when comparisons were made after 12 weeks of treatment. Similarly, the percentage of improvement in FEV<sub>1</sub> after inhalation of 5 mg salbutamol and 0.5 mg of ipratropium bromide was unchanged.

**Changes in Bronchoconstrictor Responsiveness**  
**Inhaled Histamine:** Mean log PC<sub>20</sub> values after 4, 8, and 12 weeks of treatment with budesonide or placebo are shown in Figure 1. There was an effect of period with a significant rise in log PC<sub>20</sub> (mean rise 0.18, 95 percent confidence limits 0.047 to 0.337,  $p < 0.05$ ).

Table 2—Effect of Budesonide and Placebo on Total White Blood Cell Counts, Eosinophil Counts, and IgE Levels in Venous Blood\*

	Baseline	Budesonide	Placebo
Total white blood cell counts, $\times 10^9/L$	8.99 (0.6)	8.47 (0.5)	8.8 (0.6)
Total eosinophil counts, $\times 10^9/L$	0.26 (0.04)	0.18 (0.02)	0.2 (0.0)
[----- $p = 0.03$ -----] [----- $p = 0.10$ -----]			
IgE levels, u/ml			
Log <sub>10</sub>	1.863 (0.146)	1.851 (0.146)	1.8 (0.1)
Geometric mean	73	71	64

\*Values are mean (SEM) of the 14 men. Measurements were made at recruitment (baseline) and after 12 weeks of treatment with either budesonide or placebo.



Table 3—Effect of Budesonide and Placebo on Laboratory Measurements of Spirometry, Peak Expiratory Flow, and Bronchodilator Responsiveness\*

Duration, mo	Budesonide				Placebo		
	0	1	2	3	1	2	3
FEV <sub>1</sub> , L	2.42	2.39	2.39	2.37	2.40	2.40	2.40
(pre-bd)	(0.15)	(0.16)	(0.17)	(0.17)	(0.17)	(0.17)	(0.17)
VC, L	3.96	3.70	3.69	3.69	3.85	3.80	3.77
	(0.17)	(0.16)	(0.17)	(0.18)	(0.16)	(0.16)	(0.16)
PEF, L/min	498	511	505	502	493	484	500
	(19)	(22)	(22)	(27)	(25)	(26)	(25)
ΔFEV <sub>1</sub> post-bd†	10.8			11.6			10.5
(% baseline FEV <sub>1</sub> )	(2.3)			(2.7)			(3.2)

\*Values are mean (SEM) of the 14 men.

†Increase in FEV<sub>1</sub> 1 h after inhaling a nebulized solution of 5 mg of salbutamol and 0.5 mg of ipratropium bromide.

between the end of the first period (12 weeks from start of the trial, April) and the end of the second period (24 weeks from start of trial, July). However, there was no significant effect of budesonide treatment (mean difference in log PC20 from placebo after 12 weeks of treatment 0.037, 95 percent confidence interval -0.108 to 0.182,  $p>0.5$ ) nor any evidence of drug/treatment interaction. Inspection of the mean results after four and eight weeks of budesonide or placebo again showed no significant differences or trends (Fig 1). Grand mean log PC20 for the 42 measurements with budesonide was 0.595 (SEM 0.063) and for the 42 measurements with placebo 0.591 (SEM 0.055). Attempts to detect individual smokers who responded to treatment were difficult because of the effect of season. The largest individual differences in PC20 between treatments—a mean rise of 1.18 doubling doses with budesonide and a mean rise of 1.03 doubling doses with placebo—were both found in the second half of the trial and so were in the direction of the seasonal change. In most smokers, the difference in PC20 between the two treatment periods was less than 0.5 doubling doses.

#### Peak Flow Measurements during the Last Four Weeks

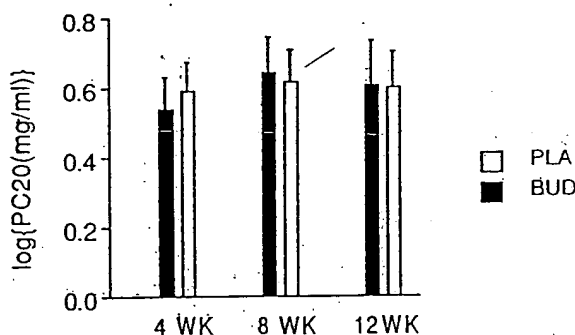


FIGURE 1. Mean (SEM) values of bronchial response to histamine (expressed as log PC20) in 14 men after 4, 8, and 12 weeks of treatment with placebo (PLA) or 1,200 µg/24 h of budesonide (BUD).

of Each Treatment (Table 4): There were no changes; diurnal variation was small throughout.

#### Single-Blind Follow-up for Nine Additional Months

**Compliance with Treatment:** Regular weighing of canisters revealed good apparent compliance in the six men taking budesonide aerosol (mean use 0.36 g/day); again with a narrow range, there was no tendency to decreased use at 12 months compared with three months.

**Untoward Events:** Three of the six men receiving budesonide had infections during the second winter of the trial and one was slow to recover so that his final measurements were not made until two months after the other men. No side effects of the drugs were encountered.

**Changes in Venous Blood:** No significant changes were observed in hemoglobin, total WBC or eosinophil counts, or in total IgE in peripheral venous blood.

**Changes in Airway Function:** At the beginning of treatment, FEV<sub>1</sub> and PEF and bronchodilator responsiveness were similar in the two groups of six men treated with budesonide and placebo, respectively (Table 5). There were no significant changes over the 12 months. Mean PEF in these men commenced at >90 percent predicted.

Analysis of the PC20 results was complicated by the

Table 4—Home PEF Values during Weeks 9 to 12 of Treatment: Budesonide or Placebo

		Budesonide		Placebo	
		Mean	(SEM)	Mean	(SEM)
AM	L/min	469	(21.0)	466	(21.4)
	%pred	95.9	(4.0)	95.4	(4.1)
PM	L/min	465	(23.4)	464	(23.1)
	%pred	95.1	(4.4)	94.8	(4.2)
Mean*		16	(2)	28	(2)
AM-PM difference					

\*Average of the mean daily AM to PM difference over four weeks in the 14 men.



Table 5—Airway Function After 12 Months of Treatment with Budesonide or Placebo\*

	Budesonide (n = 6)						Placebo (n = 6)					
	Begin		3 mo		12 mo		Begin		3 mo		12 mo	
FEV <sub>1</sub> (pre-bd), L	2.45	(0.27)	2.39	(0.22)	2.44	(0.24)	2.38	(0.35)	2.37	(0.33)	2.41	(0.33)
Bronchodilator response, %	8.6	(4.8)	10.2	(4.3)	9.7	(5.3)	11.6	(5.0)	13.6	(5.9)	10.6	(5.0)
PEF, L/min												
AM	473	(27)	479	(31)	479	(29)	453	(43)	440	(48)	438	(43)
PM	482	(28)	479	(33)	481	(33)	453	(44)	426	(50)	441	(43)
PC20 histamine, mg/ml	2.93		4.80		5.26		1.81		4.83		3.14	
Log PC20	0.467	(0.279)	0.681	(0.152)	0.721	(0.119)	0.258	(0.343)	0.684	(0.174)	0.523	(0.343)

\*Results are arithmetic mean (SEM) except for PC20 where geometric means are shown. Each PEF result is the average of the mean results obtained over four weeks of home monitoring.

effect of period in the double blind crossover trial. Thus, in the first three months with both budesonide and placebo, there was a small increase in log PC20; in the subsequent nine months, there was no further significant change in log PC20 with budesonide.

#### DISCUSSION

In the present study we were unable to demonstrate any consistent improvement in baseline lung function, home peak flow, bronchoconstrictor responsiveness, or bronchodilator responsiveness in smokers with mild airflow obstruction following three months of treatment with budesonide. These results contrast with the consistent improvement others have found with similar treatment in patients with asthma,<sup>1-11</sup> but agree with two other recent studies in smokers with chronic airways obstruction.<sup>12,13</sup>

We have considered a number of possible explanations for these negative results.

We checked compliance with treatment by regularly weighing metered dose canisters at each visit and by confirming the presence of budesonide metabolites in the blood after 12 weeks and after 12 months of treatment. In addition, we noted a significant fall in blood eosinophil count from baseline values after 12 weeks of treatment with budesonide. Although the lack of subjective benefit would be expected to reduce compliance, the men recruited into this trial were well known to us and appropriate decreases in canister weights were maintained over the full 15-month period of treatments; in the one man who withdrew from the trial, poor compliance was suspected at an early stage from canister weights. Obviously the appropriate canister weights and presence of plasma budesonide might reflect discharge of canisters and compliance in the hours before a laboratory visit; but we suspect that compliance in our men would be better than in a larger, less personally supervised trial. The dosage of budesonide chosen was the maximum we believed we could use without leading to significant impairment of hypothalamic-pituitary-adrenal control of endogenous corticosteroids. Smaller doses over a similar period

have been shown to attenuate bronchoconstrictor responsiveness in subjects with asthma.<sup>9</sup>

We found an effect of period on PC20 in our trial although we avoided studying men shortly after recognized upper or lower respiratory infection, seems probable that the attenuation of bronch responsiveness with period was associated with season. This effect of period will have tended to obscure a drug effect, but formal analysis showed no evidence of drug effect after 12 weeks' treatment or any drug-period interaction. Confirmation of the lack of effect of budesonide was obtained by comparing mean values after four and eight weeks of treatment. Short-term repeatability of PC20 in smokers appears to be similar to that in asthmatic subjects with most values falling within one doubling dilution.<sup>20</sup> The increase in PC20 induced by bronchodilators is also similar.<sup>21</sup> In the present smokers, PC20 was increased by a mean 2.3 doubling dilutions 1 h after treatment with nebulized salbutamol and ipratropium (results not shown).

The failure of budesonide to improve prebronchodilator or postbronchodilator FEV<sub>1</sub> or home PEF was a clear cut. PEF values were very close to predicted values and showed little diurnal variation so perhaps there was little room for improvement. This does not apply to FEV<sub>1</sub> where a bronchodilator effect could be demonstrated at baseline, which was quite unchanged after 12 weeks (and indeed 12 months) of treatment.

The single-blind follow-up for nine more months of treatment in two groups of six men aimed to obtain preliminary information on longer-term effects. No change was found in prebronchodilator or postbronchodilator airway function. Interpretation of the PC20 results was again complicated by the increase found in the April to June period after the first winter and by more frequent respiratory infections in the second half of the second winter. At best, however, only very small attenuation of PC20 occurred after months of treatment with budesonide.

In asthma there is a relation between PC20, histamine and the severity of asthma,<sup>22</sup> diurnal variation in PEF, and the requirement for treatment.

hence, it has been suggested that treatment should aim to attenuate BHR as well as restore normal airway function and suppress symptoms. The significance of the BHR found in smokers is less clear. In smokers there is a weaker relation of BHR to diurnal variation in PEF<sup>24</sup> and a stronger relation to baseline FEV<sub>1</sub> than in asthma, so BHR may follow rather than precede the development of airway narrowing.<sup>25</sup> Nevertheless, most previous trials of the response of baseline airway function to oral corticosteroid treatment in smokers with chronic airways obstruction have shown some improvements in at least some subjects.<sup>26,27</sup> Our failure to detect any change may reflect more rigorous exclusion of subjects with asthmatic features or the lesser degree of airways obstruction in the present subjects. Additional longer trials with a larger number of subjects will be required to assess if budesonide can slow long-term decline in lung function.

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PCT/SE98/01599

# PRV

PATENT- OCH REGISTRERINGSVERKET  
Patentavdelningen

Intyg  
Certificate



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The application was originally filed in English.

(71) Sökande Astra AB, Södertälje SE  
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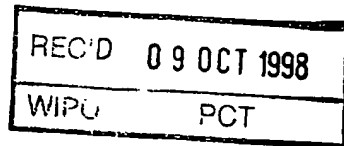
Stockholm, 1998-09-23

För Patent- och registreringsverket  
For the Patent- and Registration Office

Åsa Dahlberg

Åsa Dahlberg

Avgift  
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## PRIORITY DOCUMENT

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## NEW USE

*Field of the Invention*

The invention provides the use of formoterol and budesonide in the treatment of chronic  
5 obstructive pulmonary disease (COPD).

*Background to the Invention*

Chronic obstructive pulmonary disease (COPD) is a term which refers to a large group of  
lung diseases which can interfere with normal breathing. It is estimated that 11% of the  
10 U.S. population has COPD and the incidence is increasing. The two most important  
conditions covered by COPD are chronic bronchitis and emphysema.

Chronic bronchitis is a long-standing inflammation of the bronchi which causes increased  
production of mucous and other changes. The patients' symptoms are cough and  
15 expectoration of sputum. Chronic bronchitis can lead to more frequent and severe  
respiratory infections, narrowing and plugging of the bronchi, difficult breathing and  
disability.

Emphysema is a chronic lung disease which affects the alveoli and/or the ends of the  
20 smallest bronchi. The lung loses its elasticity and therefore these areas of the lungs become  
enlarged. These enlarged areas trap 'stale' air and do not effectively exchange it with fresh  
air. This results in difficult breathing and may result in insufficient oxygen being delivered  
to the blood. The predominant symptom in patients with emphysema is shortness of  
breath.

25 At present COPD is treated with a variety of inhaled or orally administered  
bronchodilators, with inhaled anti-cholinergic agents and with orally administered steroids.  
The problem with these treatments is that none of them are especially effective.  
Accordingly a new treatment is required.

*Description of the Invention*

It has surprisingly been found that the combination of formoterol and budesonide is unexpectedly effective in treating COPD.

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According to the invention there is provided the use of a composition comprising, in admixture:

(a) a first active ingredient which is formoterol, a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt; and

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(b) a second active ingredient which is budesonide;

in the manufacture of a medicament for use in the treatment of chronic obstructive pulmonary disease.

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The composition used in the invention optionally additionally comprises one or more pharmaceutically acceptable additives, diluents and/or carriers. The composition is preferably in the form of a dry powder, the particles of which preferably have a mass median diameter of less than 10  $\mu\text{m}$ .

The invention also includes the use of a kit containing:

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(i) a vessel containing the first active ingredient;

(ii) a vessel containing the second active ingredient; and

(iii) instructions for the sequential or separate administration of the active ingredients to a patient in need thereof;

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in the manufacture of a medicament for use in the treatment of chronic obstructive pulmonary disease.

A patient suffering from COPD can be treated by administering via inhalation a composition as defined above. Alternatively such a patient can be treated by administering via inhalation, sequentially or separately, (i) a dose of the first active ingredient; and (ii) a

dose of the second active ingredient. The doses can be provided to the patient for inhalation in dry powder form.

The invention further provides the use of budesonide and of formoterol in the manufacture of a composition or a kit, as used in the invention, for use in the treatment of chronic obstructive pulmonary disease.

The first and second active ingredients of the kit used in the invention can be administered sequentially or separately to treat respiratory disorders. By sequential is meant that the first and second active ingredients are administered one immediately after the other. They still have the desired effect if they are administered separately but less than about 12 hours apart, preferably less than about 2 hours apart, more preferably less than about 30 minutes apart.

The molar ratio of the first active ingredient to the second active ingredient in the invention is preferably from 1:2500 to 12:1, more preferably from 1:555 to 2:1, most preferably from 1:133 to 1:6.

Preferably the amount of the first active ingredient used is preferably from 2 to 120 nmol (more preferably from 7 to 70 nmol). The amount of the second active ingredient used is preferably from 0.1 to 5  $\mu$ mol (preferably 0.15 to 4  $\mu$ mol) or from 45 to 2200  $\mu$ g, more preferably from 65 to 1700  $\mu$ g.

Suitable physiologically acceptable salts of formoterol include acid addition salts derived from inorganic and organic acids, for example the chloride, bromide, sulphate, phosphate, maleate, fumarate, tartrate, citrate, benzoate, 4-methoxybenzoate, 2- or 4-hydroxybenzoate, 4-chlorobenzoate, p-toluenesulphonate, methanesulphonate, ascorbate, acetate, succinate, lactate, glutarate, gluconate, tricarallylate, hydroxynaphthalene-carboxylate or oleate salts or solvates thereof. The first active ingredient is preferably formoterol fumarate, especially the dihydrate.

When the first active ingredient is formoterol fumarate dihydrate, the amount of the first active ingredient used is preferably from 1 to 50  $\mu\text{g}$ , more preferably from 3 to 30  $\mu\text{g}$ .

- 5 More preferably the composition or kit used in the invention comprises 6 $\mu\text{g}$  of formoterol fumarate dihydrate and 100 $\mu\text{g}$  of budesonide, or 4.5 $\mu\text{g}$  of formoterol fumarate dihydrate and 80 $\mu\text{g}$  of budesonide, either of which is administered up to four times a day.
- Alternatively the composition or kit of the invention comprises 12 $\mu\text{g}$  of formoterol fumarate dihydrate and 200 $\mu\text{g}$  of budesonide, or 9 $\mu\text{g}$  of formoterol fumarate dihydrate and
- 10 160 $\mu\text{g}$  of budesonide, either of which is administered once or twice a day.

Most preferably the composition or kit used in the invention comprises 6 $\mu\text{g}$  of formoterol fumarate dihydrate and 200 $\mu\text{g}$  of budesonide, or 4.5 $\mu\text{g}$  of formoterol fumarate dihydrate and 160 $\mu\text{g}$  of budesonide, either of which is administered up to four times a day.

- 15 Alternatively the composition or kit of the invention comprises 12 $\mu\text{g}$  of formoterol fumarate dihydrate and 400 $\mu\text{g}$  of budesonide, or 9 $\mu\text{g}$  of formoterol fumarate dihydrate and 320 $\mu\text{g}$  of budesonide, either of which is administered once or twice a day.

Preferably the active ingredient(s) are used in admixture with one or more

- 20 pharmaceutically acceptable additives, diluents or carriers, preferably in an amount of from 50 $\mu\text{g}$  to 25mg per dose, more preferably in an amount of from 50 $\mu\text{g}$  to 10mg, most preferably in an amount of from 100 to 2000 $\mu\text{g}$ . Examples of suitable diluents or carriers include lactose, dextran, mannitol or glucose. Preferably lactose is used, especially as the monohydrate.

25 One or more of the ingredients is preferably in the form of a dry powder, more preferably a micronised dry powder, most preferably an agglomerated micronised dry powder. As an alternative to agglomeration, the finely divided active ingredients may be in the form of an ordered mixture with the pharmaceutically acceptable additive, diluent or carrier. An

30 ordered mixture comprises fine particles of an active ingredient in association with coarse

particles of the pharmaceutically acceptable additive, diluent or carrier. The ingredients used in the invention can be obtained in these preferred forms using methods known to those of skill in the art. The particle size of the active ingredients is preferably less than 10 $\mu$ m.

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Administration may be by inhalation orally or intranasally. The active ingredients are preferably adapted to be administered, either together or individually, from dry powder inhaler(s), especially the Turbuhaler<sup>®</sup> (Astra AB), pressurised metered dose inhaler(s), or nebuliser(s).

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When the active ingredients are adapted to be administered, either together or individually, from pressurised inhaler(s), they are preferably in micronised form. They are dissolved or, preferably, suspended in a liquid propellant mixture. The propellants which can be used include chlorofluorocarbons, hydrocarbons or hydrofluoroalkanes. Especially preferred propellants are P134a (tetrafluoroethane) and P227 (heptafluoropropane) each of which may be used alone or in combination. They are optionally used in combination with one or more other propellants and/or one or more surfactants and/or one or more other excipients, for example ethanol, a lubricant, an anti-oxidant and/or a stabilising agent.

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When the active ingredients are adapted to be administered, either together or individually, via nebuliser(s) they may be in the form of a nebulised aqueous suspension or solution, with or without a suitable pH or tonicity adjustment, either as a unit dose or multidose device.

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The composition or kit used in the invention may optionally be administered as divided doses from 1 to 4, and preferably once or twice a day.

The invention is illustrated by the following Examples which are not intended to limit the scope of the application. In the Examples micronisation is carried out in a conventional



manner such that the particle size range for each component is suitable for administration by inhalation. Turbuhaler is a trademark of Astra AB.

*Example 1*

5 6 Parts by weight of formoterol fumarate dihydrate was mixed with 794 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. 200 Parts by weight of micronised budesonide was added to the conditioned product by mixing and homogenising with a low pressure jet mill. The mixture was then spheronised using the process of EP-A-721 331  
10 and filled into the storage compartment of a Turbuhaler.

*Example 2*

4.5 Parts by weight of formoterol fumarate dihydrate was mixed with 835 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and  
15 then conditioned using the process of EP-A-717 616. 160 Parts by weight of micronised budesonide was added to the conditioned product by mixing and homogenising with a low pressure jet mill. The mixture was then spheronised using the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

*Example 3*

20 12 Parts by weight of formoterol fumarate dihydrate was mixed with 588 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. 400 Parts by weight of micronised budesonide was added to the conditioned product by mixing and homogenising with a low  
25 pressure jet mill. The mixture was then spheronised using the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

*Example 4*

6 Parts by weight of formoterol fumarate dihydrate was mixed with 894 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. 100 Parts by weight of micronised  
5 budesonide was added to the conditioned product by mixing and homogenising with a low pressure jet mill. The mixture was then spheronised using the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

*Example 5*

10 4.5 Parts by weight of formoterol fumarate dihydrate was mixed with 915 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. 80 Parts by weight of micronised budesonide was added to the conditioned product by mixing and homogenising with a low pressure jet mill. The mixture was then spheronised using the process of EP-A-721 331  
15 and filled into the storage compartment of a Turbuhaler.

*Example 6*

12 Parts by weight of formoterol fumarate dihydrate was mixed with 788 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and  
20 then conditioned using the process of EP-A-717 616. 200 Parts by weight of micronised budesonide was added to the conditioned product by mixing and homogenising with a low pressure jet mill. The mixture was then spheronised using the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

*Example 7*

25 6 Parts by weight of formoterol fumarate dihydrate was mixed with 994 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. The mixture was then spheronised using the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

200 Parts by weight of micronised budesonide was mixed with 800 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. The mixture was then spheronised using the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

#### Example 8

4.5 Parts by weight of formoterol fumarate dihydrate was mixed with 995 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. The mixture was then spheronised using the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

160 Parts by weight of micronised budesonide was mixed with 840 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. The mixture was then spheronised using the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

#### Example 9

12 Parts by weight of formoterol fumarate dihydrate was mixed with 988 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. The mixture was then spheronised using the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

400 Parts by weight of micronised budesonide was mixed with 600 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. The mixture was then spheronised using the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

*Example 10*

6 Parts by weight of formoterol fumarate dihydrate was mixed with 994 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. The mixture was then spheronised using  
5 the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

100 Parts by weight of micronised budesonide was mixed with 900 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. The mixture was then spheronised using  
10 the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

*Example 11*

4.5 Parts by weight of formoterol fumarate dihydrate was mixed with 995 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and  
15 then conditioned using the process of EP-A-717 616. The mixture was then spheronised using the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

80 Parts by weight of micronised budesonide was mixed with 920 parts by weight of  
20 lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. The mixture was then spheronised using the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

*Example 12*

25 12 Parts by weight of formoterol fumarate dihydrate was mixed with 988 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. The mixture was then spheronised using the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

200 Parts by weight of micronised budesonide was mixed with 800 parts by weight of lactose monohydrate. The blend was micronised using a high pressure air jet mill and then conditioned using the process of EP-A-717 616. The mixture was then spheronised using the process of EP-A-721 331 and filled into the storage compartment of a Turbuhaler.

*Claims*

1. Use of a composition comprising, in admixture:
  - (a) a first active ingredient which is formoterol, a pharmaceutically acceptable salt or  
5 solvate thereof, or a solvate of such a salt; and
  - (b) a second active ingredient which is budesonide;in the manufacture of a medicament for use in the treatment of chronic obstructive pulmonary disease.
- 10 2. Use according to claim 1, wherein the composition comprises one or more pharmaceutically acceptable additives, diluents and/or carriers.
3. Use of a kit containing:
  - (i) a vessel containing a first active ingredient which is formoterol, a pharmaceutically  
15 acceptable salt or solvate thereof, or a solvate of such a salt;
  - (ii) a vessel containing a second active ingredient which is budesonide; and
  - (iii) instructions for the sequential or separate administration of the first and second active ingredients to a patient in need thereof;in the manufacture of a medicament for use in the treatment of chronic obstructive  
20 pulmonary disease.
4. Use according to claim 3, wherein the first and/or second active ingredient is used in admixture with one or more pharmaceutically acceptable additives, diluents and/or carriers.
- 25 5. Use according to any one of the preceding claims, wherein the first active ingredient is formoterol fumarate dihydrate.
6. Use according to any one of the preceding claims, wherein the molar ratio of the first active ingredient to the second active ingredient is from 1:2500 to 12:1.

7. Use of formoterol, a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt in the manufacture of a composition as defined in claim 1 or 2 or a kit as defined in claim 3 or 4 for use in the treatment of chronic obstructive pulmonary disease.

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8. Use of budesonide in the manufacture of a composition as defined in claim 1 or 2 or a kit as defined in claim 3 or 4 for use in the treatment of chronic obstructive pulmonary disease.

10 9. A method for the treatment of a patient suffering from chronic obstructive pulmonary disease which method comprises administering to the patient via inhalation, sequentially or separately, a therapeutically effective amount of (i) a dose of a first active ingredient which is formoterol, a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt; and (ii) a second active ingredient which is budesonide;

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10. A method for the treatment of a patient suffering from chronic obstructive pulmonary disease which method comprises administering to the patient via inhalation a therapeutically effective amount of a composition as defined in claim 1 or 2.

Patent 1,100,100

*Abstract*

The invention provides the use of formoterol and budesonide in the treatment of chronic obstructive pulmonary disease.

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